From:	Dunbar, Anwar
To:	Gallagher, Sarah
Subject:	Cancer Studies
Date:	Thursday, May 14, 2015 2:52:00 PM
Attachments:	47841985.der.doc 47841987.der.doc 48306965.der.docx 48306966.der.docx

---

Here you go Sarah. These are some of my favorites☺ Pyrifluqinazon and bicyclopyrone were both mine. Notice how the rat and the mouse react to the chemicals. You may see similar effects with benzobicyclone to bicyclopyrone.

Anwar Y. Dunbar, Ph.D., Pharmacologist
Risk Assessment Branch 1
The Human Health Effects Division/ The Office of Pesticide Programs
1200 Pennsylvania Ave, NW
Washington, DC 20460

"Except for in the most unique of circumstances, mastery of any cognitively complex skill or task requires roughly 10,000 hours of practice"- Malcolm Gladwell, Author of the book Outliers

EPA Reviewer: Anwar Dunbar, Ph.D. **Signature:** _____
Risk Assessment Branch I, Health Effects Division (7509P) Date: _____
EPA Reviewer: Greg Akerman, Ph.D. **Signature:** _____
Risk Assessment Branch I, Health Effects Division (7509P) Date: _____

TXR#: 0057111

DATA EVALUATION RECORD

PC CODE: 018986 **DP BARCODE:** D425155

STUDY TYPE: Carcinogenicity and combined 52 week toxicity study – Rat (feeding)

OECD 453 (2009): OPPTS 870.4300 (1998): EU Directive 96/64/EEC B.33 (2001): JMAFF No. 12-Nohsan-8147 (2000)

TEST MATERIAL (PURITY): NOA449280 (94.5% purity)

SYNONYMS: Bicyclo[3.2.1]oct-3-en-2-one,4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-(trifluoromethyl)-3-pyridinyl]carbonyl]-; 4-hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-(trifluoromethyl)-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one; bicyclopyrone, SYN449280.

CITATION: Robertson B and Perry C, 2012. NOA449280: 104 week rat dietary carcinogenicity study with combined 52 week toxicity study. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 30197, 31 August 2012. (Syngenta File No.NOA449280_11302). MRID 47841985

SPONSOR: Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, United Kingdom.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In a combined 52 week chronic/104 week rat dietary carcinogenicity study (MRID #47841985), bicyclopyrone (94.5% purity) was administered to groups of Han Wistar (CrL:WI(Han)) rats in the diet. Five groups of 52 male and 52 female Han Wistar rats were assigned to the Carcinogenicity study and dosed with diets containing 0, 5, 500, 2500 or 5000 ppm bicyclopyrone for at least 104 consecutive weeks. In addition, a chronic toxicity study comprising a further 5 groups of 12 males and 12 females was included and dosed in an identical fashion for a period of 52 consecutive weeks. The equivalent doses for the carcinogenicity phase of the study were 0, 0.28/0.35, 28.4/35.8, 141/178 and 280/368 mg/kg/day (M/F). The equivalent doses for the chronic toxicity phase of the study were 0, 0.32/0.39, 32.6/41.6, 166/204 and 335/404 mg/kg/day (M/F). Since the achieved doses are similar between the two phases, the doses from the carcinogenicity phase will be used for risk assessment purposes.

Due to observation of severe eye lesions, corneal opacity and damage, 5000 ppm male animals were fed blank control diet over a 9 day period during Weeks 4 and 5. Dosing recommenced after this period and was continuous to the end of the study.

The following were assessed at pre-determined intervals from pre-trial until study completion from carcinogenicity and chronic toxicity study animals: clinical observations, body weight, food consumption, haematology, coagulation and clinical chemistry. Additionally, selected carcinogenicity study animals had samples taken for urinalysis at predetermined intervals and all underwent ophthalmoscopy examinations prior to initiation of dosing and at weeks 50 and 102. Toxicity study animals received a detailed functional observation battery assessment once during treatment (weeks 51/52).

All surviving Carcinogenicity and Toxicity study animals were terminated and subjected to a detailed necropsy examination with a comprehensive histological evaluation after the completion of 104 or 52 weeks of treatment respectively.

The effects are as follows:

There were no statistically significant differences in mortality between the controls and any groups treated with bicyclopyrone.

At 5 ppm bicyclopyrone, there was a 2-6% increase in the incidence of opaque eyes and corneal damage in both sexes compared to the control group (0-2%). At 104 weeks in males, there was an increased incidence of thyroid follicular hyperplasia in males (19%) compared to the control group (4%). There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (63%) compared to the control group (33%).

At 500 ppm bicyclopyrone, there was a significant increase in the incidence of opaque eyes and corneal damage in both sexes (98-100%) compared to controls (0-2%). There was an increase in the incidence of eye keratitis (88-100% for males and 87-100% for females) and the regenerative corneal hyperplasia (88-100% for males and 42-92% for females) from 52 weeks to 104 weeks compared to the control group (2%). In males, there was an increased incidence of thyroid follicular hypertrophy (75%) at 52 weeks compared to the control group (0%). At 104 weeks in males, there was an increased incidence of thyroid follicular hyperplasia (23%) compared to the control group (19%). This effect occurred in females as well but there was no dose response. There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (75%) compared to the control group (33%). In males, there was an increased incidence of squamous cell carcinoma and papilloma (4% and 2%) compared to the control group (0%).

At 2500 ppm bicyclopyrone, there was a significant increase in the incidence of opaque eyes and corneal damage in both sexes compared to controls (98-100%) compared to the control group (0-2%). Decreases in absolute body weights for females were transiently statistically significant through the study (↓5-10%). Relative to the control group, there was a minor decrease in the absolute brain weights of males and females (↓3-7%), and heart weights of females (↓7%). There was an increase in the incidence of eye keratitis (83% for males and 87-92% for females) and regenerative corneal hyperplasia (58-63% for males and 58% for females) from 52 weeks to 104 weeks compared to the control group (2%). In males, there was an increased incidence of thyroid follicular hypertrophy (83%) at 52 weeks compared to

the control group (0%). At 104 weeks in males, there was an increased incidence of thyroid follicular hyperplasia (23%) compared to the control group (4%). There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (77%) compared to the control group (33%). There was a statistically significant increase in the incidence of acinar cell atrophy in the pancreas of male animals (50%) compared to the control group (27%). In males, there was an increased incidence of squamous cell carcinoma and papilloma (4% and 2%) compared to the control group (0%).

At 5000 ppm bicyclopyrone, there was a significant increase in the incidence of opaque eyes and corneal damage in both sexes (98-100%) compared to the control group (0-2%). Relative to the control group, in both sexes there were significantly lower body weights (↓5-16% for males and ↓5-20% for females). There were also minor changes in food consumption and utilization. There was an increase in the incidence of eye keratitis (88-100% for males and 73-92% for females) and the regenerative corneal hyperplasia (71-100% for males and 35-75% for females) from 52 weeks to 104 weeks compared to the control group (2%). In males, there was an increased incidence of thyroid follicular hypertrophy (66%) at 52 weeks compared to the control group (0%). At 104 weeks in males, there was an increased incidence of thyroid follicular hyperplasia (33%) compared to the control group (4%). There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (69%) at 104 weeks compared to the control group (33%). There was a statistically significant increase in the incidence of acinar cell atrophy in the pancreas of male animals (58%) compared to the control group (27%). In males, there was an increased incidence of squamous cell carcinoma and papilloma (4% and 6%) compared to the control group (0%).

The corneal tumors seen in males rats are associated with and likely attributable to significant damage to and regenerative hyperplasia of the cornea seen during the course of the carcinogenicity study with bicyclopyrone at concentrations of 500 ppm and above. The identified mode of action of HPPD inhibiting herbicides results in significantly elevated plasma tyrosine in rats, particularly males. EPA's Cancer Assessment Review Committee determined that in male rats, there was a dose-dependent increase in corneal tumors which were considered treatment related (Rowland et al., September 10, 2014, TXR #0057011). The doses tested were considered to be adequate and not excessive, for assessing carcinogenicity in both sexes. This was based upon increases in corneal opacity, decreased absolute body weights in both sexes at the high dose, and an increased incidence of regenerative corneal hyperplasia in both sexes.

Based upon the effects in this study, the LOAEL for systemic toxicity is 5 ppm (0.28/0.35 mg/kg/day [M/F]) based on a dose dependent increase in the incidence of opaque eyes and corneal damage in both sexes compared to controls, an increased incidence of thyroid follicular hyperplasia in males, and an increased incidence of chronic progressive nephropathy in the kidneys of males. The NOAEL was not established.

This study is classified as totally reliable **(acceptable/guideline)** as a combined chronic/carcinogenicity study in rats (OPPTS 870.4300; OECD 451). EPA, PMRA, and AMPVA agree on the regulatory decision and classification for this study.

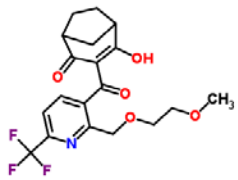
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging and Quality Assurance statements were provided.

MATERIALS AND METHODS

Materials:

Test Material:	Bicyclopyrone (NOA449280)
Description:	Technical, solid beige powder
Lot/Batch number:	SEZ3AP006/MILLED
Purity:	94.5% a.i
CAS#:	352010-68-5
Stability of test compound:	Stable (stored at a temperature < 30°C; light protected, dry)

Structure:



Vehicle and/or positive control: The test substance was administered via Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) (Special Diets Services Limited, 1 Stepfield, Witham, Essex, UK)).

Test Animals:	
Species	Rat
Strain	Han Wistar (CrL:WI(Han))
Age/weight at dosing	Approximately 6 weeks / 104-184 g (males), 96-155 g (females)
Source	Charles River UK Limited, Margate, Kent, UK
Housing	Up to 4 per cage by sex and dose group, in suspended polycarbonate cages (overall dimensions 59 x 38.5 x 20 cm) with stainless steel grid tops and food hopper.
Acclimatisation period	13 days
Diet	Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) (Special Diets Services Limited, 1 Stepfield, Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 9.41-23.69°C (target range 19-23°C) Humidity: 13.87-85.06% (target range 40-70%) Air changes: Minimum of 15 air changes per hour Photoperiod: 12 hrs dark / 12 hrs light

In-life dates: Start: 30 April 2007 End: 20 October 2009

Study Design and Methods: In a 104 week rat dietary carcinogenicity study with combined 52 week chronic toxicity study, NOA449280 (94.5% purity) was administered to groups of Han Wistar (CrL:WI(Han)) rats in the diet.

Carcinogenicity study animals (52/sex/dose) were dosed continuously by the diet for at least 104 weeks and chronic toxicity study animals (12/sex/dose) for at least 52 weeks with the exception of 5000 ppm males where the animals were removed from treatment and given

blank diet on 25 May 2007 due to eye lesions. Dosing recommenced for these animals on the 04 June 2007.

Animal assignment: On arrival from the suppliers, the animals were introduced to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Each group in the carcinogenicity study was housed on a separate rack. In the chronic toxicity study, control animals were housed on the same rack as treated groups. Each month, from the commencement of pretrial, each column of cages on a rack was moved one position to the right. During pretrial, group mean body weights were checked to ensure that all groups had a similar body weight for each sex and were all found to be within a 20% limit of variation. Animals were allocated to dose groups as in the table below:

Table 1: Study design

Test group	Dietary concentration (ppm)	Animal numbers			
		Carcinogenicity study		Chronic toxicity study	
		males	females	males	females
Control	0	1-52	261-312	521-532	581-592
Low	5	53-104	313-354	533-544	593-604
Intermediate 1	500	105-156	365-416	545-556	605-616
Intermediate 2	2500	157-208	417-468	557-568	617-628
High	5000	209-260	469-520	569-580	629-640

*Table was taken from page 22 of the study report

Diet preparation and analysis: Control, 500, 2500 and 5000 ppm diets were made and dispensed weekly for administration to the animals. 5 ppm diets were made weekly, and stored frozen at -20°C until required. Diet preparations were made as a serial dilution from a stock of the high dose level of 5000 ppm. The stock was prepared by mixing test item with the required amount of untreated control diet in an automated mortar and pestle and ground for 5 min. The premix was then blended with the required amount of untreated diet and mixed for 20 minutes in a diet mixer (Winkworth). The diets at the lower concentrations (5, 500, 2500 and an intermediate level of 100 ppm) were prepared as a serial dilution from the higher concentration group by adding an appropriate amount of untreated diet. Diets were mixed for 20 minutes in a Winkworth change drum mixer. The diets were stored at ambient room temperature (except for the 5 ppm dose up until week 12 which was stored -20ºC in the dark).

Prior to study commencement, stability data was generated by Charles River, Edinburgh for diet preparations stored at -20°C for 15 days and for 7 days (500-5000 ppm) or 1 day (5 ppm) at ambient laboratory temperature. During the study, triplicate samples (3 x 50 g) were taken from each diet (including control) at approximately 3 monthly intervals immediately after preparation and analysed for concentration and homogeneity.

Concentration analysis results: Analysed concentrations of test item within the diet were generally found to be within ±8.2% of the theoretical concentrations.

Homogeneity results: The Week 1 low dose group (5 ppm) was out of the acceptable criteria for homogeneity (CV ≤37%) and was re-analyzed. Subsequent analyses for all samples were with the acceptable range (CV <12.3%).

Stability results: Stability analyses revealed differences in concentrations ranging from 2.4-13.6% of the theoretical, with coefficients of variation ranging from 0.3-8.4%. Deviations were isolated and were considered to be minimal and not to have affected the outcome or integrity of the study. Bicyclopyrone was not detected in the control diet.

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable, provided that the cited stability study did indicate that the test compound was stable under conditions of the study.

Observations: All animals were checked twice per day for signs of viability. Once each week all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Body weight: The body weight of each rat was recorded once weekly during pre-trial up until Week 14 of treatment, and once every 2 weeks from week 16 up until the end of treatment.

Food consumption and test substance intake: The quantity of food consumed by each cage of animals was measured and recorded once weekly during pre-trial up until week 14 of treatment, and once every 4 weeks from week 16 up until the end of treatment. Food utilization was calculated for weeks 1-4, 5-8, 9-13 and 1-13, according to the following formula:

(Cage mean weight gain x 100) / cage total food consumption.

The amount of test item ingested was calculated at regular intervals during treatment using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{Nominal Concentration (ppm)} \times \text{Food Consumption (g/day)}}{\text{Mid-point Body Weight (g)}}$$

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Ophthalmoscopic examination: Eyes were examined using an indirect ophthalmoscope following application of a mydriatic agent (1% Tropicamide, Mydriacyl®). The cornea, anterior chamber, iris, lens, posterior chamber, retina and vessels of the optic disc were examined from all Carcinogenicity animals during pre-trial and weeks 50 and 102.

Functional observation battery: Once during the treatment period (week 51/52) a more detailed examination was made of all chronic toxicity study animals. The examinations were made by a technician not involved in the dosing procedures or in the collection of body weights and food consumption data, and were performed at an approximately standardised time of day. Prior to the independent technician entering the room, standard cage cards were removed and only neurotoxicity cards were shown. The assessor was then allowed to enter the room. Three animals from each cage had their tail marked for identification purposes. The following were assessed:

Cage side observations: Prostration, lethargy, writhing, circling, breathing abnormalities, gait abnormalities, tremor, fasciculation, convulsions, biting (of cage components or self mutilating), vocalisations, piloerection, ease of removal from the cage, body temperature (taken directly by from the implanted electronic chip and recorded), condition of the eyes (checked for: pupillary function, miosis, mydriasis, exophthalmos, encrustation, lachrymation), condition of the coat, presence of salivation, overall ease of handling.

Observations in a standardised area (2 min observation): Latency (time to first locomotory movement), level of mobility, rearing, grooming, urination/defecation, arousal (level of alertness), posture, tremor/convulsions, vocalisation, piloerection, palpebral closure, gait abnormalities, stereotypy (excessive repetition of behaviours) and/or unusual behaviours.

Functional Tests: Once during the treatment period (week 51/52), the following additional functional tests were performed: Reaction to sudden sound (click above the head), reaction to touch on the rump with a blunt probe, grip strength, pain perception, landing foot splay.

Motor activity: Each animal was placed in an individual monitoring cage, scanned by a motion sensor utilising infra-red pyroelectric detectors. Movement was detected in 3 dimensions anywhere in the cage, and was differentiated into large and small movements. Each animal was monitored for one session with movement recording at 5-minute intervals, and each session was run for at least 1 hour (between 71–84 minutes).

Clinical pathology: Blood samples for haematology (0.5 mL, into EDTA tubes), coagulation (0.5 mL, into 0.045 mL trisodium citrate tubes) and clinical chemistry (1.5 mL, into lithium heparin tubes) were obtained, *via* the tail vein and without anaesthesia, from 13 males and 13 females per group from the carcinogenicity study at weeks 14, 27, 53 and 79, all surviving carcinogenicity study animals during weeks 104, and all surviving Toxicity study animals during Week 52. The animals were not deprived of food overnight prior to sampling.

Haematology and coagulation: The following parameters were examined:

haemoglobin
haematocrit
red blood cell count
mean cell volume
mean cell haemoglobin
prothrombin time
mean cell haemoglobin concentration
platelet count
total white cell count
differential white cell count
activated partial thromboplastin time

Clinical chemistry: The following parameters were examined:

urea
creatinine
glucose
albumin
total protein
cholesterol
triglycerides
total bilirubin
creatine phosphokinase
globulin
alkaline phosphatase activity
aspartate aminotransferase activity
alanine aminotransferase activity
gamma-glutamyl transferase activity
calcium
phosphate
sodium
potassium
chloride
AG ratio

Urinalysis: Urine samples were collected over a 4 h period from 13 male and 13 female carcinogenicity study animals per group during Weeks 13, 26, 52, 78 and 103. The animals were housed individually in metabolism cages and were deprived of food and water. The following parameters were evaluated:

volume
colour
specific gravity
pH
urobilinogen
microscopy of spun deposit
glucose
ketones
protein
bilirubin
blood pigments

Investigations *post mortem*: After at least 52 or 104 weeks of treatment all surviving animals were killed in random order by exposure to carbon dioxide and had their terminal body weight recorded, followed by exsanguination.

Macroscopic examination: All animals were examined *post mortem*. This consisted of a complete external and internal examination which included body orifices (ears, nostrils, mouth, anus, vulva) and cranial, thoracic and abdominal organs and tissues.

Organ weights: From all chronic toxicity and carcinogenicity animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands
brain
epididymides
heart
kidneys
ovaries
spleen
testes
liver
uterus (with oviducts)

Paired organs were weighed together.

Tissue submission: The following tissues from all chronic toxicity and carcinogenicity animals, surviving to scheduled termination, were examined *in situ*, removed and processed top paraffin wax blocks, stained with haematoxylin and eosin and examined histopathologically:

gross lesions including masses (with lymph nodes local to masses)
adrenal gland
aortic arch
brain (forebrain, midbrain, cerebellum, pons)
bone marrow (femur)
caecum
colon
duodenum
epididymis
eyes (including optic nerve)
oesophagus
ovary
oviduct
Peyer’s patches
pancreas
parathyroid gland
pharynx
pituitary gland
prostate gland
rectum

Harderian gland
heart
ileum
jejunum
kidney
lachymal gland
larynx
liver
lung
lymph node – mesenteric and submandibular
thigh muscle
tongue
uterus
vagina
salivary gland
seminal vesicle
spinal cord (cervical, mid thoracic, lumbar)
skin and mammary gland
spleen
sternum
sciatic nerve
stomach
testis
thymus
thyroid gland
trachea
urinary bladder

Microscopic examination: All processed tissues were 4-6 µm thick and were examined by light microscopy.

Statistics: Body weight, cumulative body weight gain, food consumption, food utilization, haematology, coagulation, clinical chemistry, quantitative urinalysis values, quantitative functional observation battery measurements, motor activity and organ weight data were analysed using a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test. The following pairwise comparisons were performed: Control Group vs Low Dose, Control Group vs Intermediate Dose, Control Group vs High Dose. Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as covariate. Kaplan-Meier survival estimates were calculated separately for each sex and treatment group. Histological incidence data and pairwise comparisons of the incidence of tumor and histological lesions were made using Fisher's Exact test. Further analysis was performed using Peto's time adjusted methods. Methods used for the age-adjusted analysis of fatal and non-fatal tumors were based on the IARC guidelines.

RESULTS AND DISCUSSION

Mortality: There was no statistically significant difference in mortality between the control and any other groups for males or females.

Clinical observations: Almost all males and females treated at 500 ppm and above were noted to have opaque eyes and corneal damage (≥98% of animals in these groups). The distribution was equal between both eyes. This finding was generally seen from approximately Week 4 until the end of the treatment period. See table 2.

Table 2: Intergroup comparison of the incidence of opaque eyes and corneal damage

Finding	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Eyes opaque										
Number of animals	1 (2%)	3 (6%)	51 (98%)	51 (98%)	51 (98%)	0	1 (2%)	52 (100%)	52 (100%)	50 (100%)

Days from - to	408-737	387-737	24-737	23-737	23-737		640-710	24-738	24-738	24-738
Corneal damage										
Number of animals	0	1 (2%)	51 (98%)	51 (98%)	51 (98%)	0	0	51 (98%)	52 (100%)	50 (96%)
Days from-to		352-359	24-737	24-737	24-737			24-738	24-738	24-738

Data were taken from page 43 of the study report

Body weight and weight gain: Significantly lower absolute body weight was seen in both sexes throughout the study at 2500 (↓5-7% for males and ↓4-10% for females) and 5000 ppm (↓5-10% for males and ↓6-20% for females). Decreases for males at 500 ppm were not statistically significant. See table 3.

Table 3: Mean intergroup comparison of absolute bodyweights (g) by week

	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
Week*	**0**	**5**	**500**	**2500**	**5000**	**0**	**5**	**500**	**2500**	**5000**
Pre-Test (Week 0)	143.8 ± 14.1	139.3 ± 16.2	137.7 ± 17.3	147.3 ± 16.1	146.1 ± 16.1	122.5 ± 12.4	125.5 ± 12.7	127.8* ± 9.9 (↑4%)	125.4* ± 12.0 (↑2%)	128.8** ± 10.5 (↑5%)
Week 2	213.4 ± 23.3	223.3 ± 21.0	201.0 ± 27.7	213.7 ± 18.1	203.0* ± 23.0 (↓5%)	158.2 ± 14.9	168.9** ± 14.7 (↑6%)	164.7* ± 11.7 (↑4%)	159.9 ± 12.1	160.1 ± 12.5
Week 7	332.5 ± 32.9	340.5 ± 28.7	320.3 ± 28.9	312.7** ± 24.0 (↓6%)	299.9** ± 30.8 (↓10%)	216.2 ± 17.7	220.3 ± 18.7	215.5 ± 15.5	208.6* ± 16.6 (↓4%)	203.9* ± 14.5 (↓6%)
Week 13	377.6 ± 38.9	386.3 ± 35.6	365.6 ± 34.1	357.1** ± 33.8 (↓5%)	345.6** ± 36.9 (↓8%)	237.1 ± 18.8	241.1 ± 21.5	236.2 ± 16.9	229.9 ± 18.8	225.3** ± 16.8 (↓5%)
Week 26	440.4 ± 42.1	448.9 ± 44.0	422.5 ± 41.7	412.0** ± 37.9 (↓6%)	403.7** ± 44.8 (↓8%)	261.9 ± 20.7	261.7 ± 22.6	257.4 ± 21.1	250.2** ± 20.4 (↓4%)	243.4** ± 17.2 (↓7%)
Week 52	529.4 ± 50.8	540.8 ± 59.9	511.7 ± 56.5	499.4** ± 52.8 (↓6%)	487.6** ± 47.0 (↓8%)	301.9 ± 40.0	306.2 ± 37.7	298.6 ± 40.6	289.7 ± 39.0	267.4** ± 23.0 (↓11%)
Week 78	583.2 ± 67.6	603.5 ± 80.8	574.4 ± 70.1	551.1 ± 64.9	541.6* ± 56.8 (↓7%)	366.9 ± 53.2	377.1 ± 54.5	362.2 ± 59.4	343.4 ± 54.8	303.5** ± 34.3 (↓17%)
Week 96	612.3 ± 69.3	627.6 ± 91.7	594.3 ± 74.4	568.8* ± 59.0 (↓7%)	565.9* ± 59.0 (↓8%)	403.6 ± 52.6	399.2 ± 60.8	397.3 ± 64.6	366.1* ± 58.8 (↓9%)	323.8** ± 39.4 (↓20%)
Week 104	610.8 ± 74.9	635.8 ± 89.8	593.0 ± 74.2	569.6* ± 63.4 (↓7%)	561.5* ± 61.5 (↓8%)	406.5 ± 59.6	408.8 ± 69.5	405.9 ± 67.2	367.0* ± 58.9 (↓10%)	328.4** ± 45.2 (↓19%)

Data were taken from pages 88-97 of the study report

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Significantly lower body weight gains were seen in both sexes throughout the study at 2500 (↓8-14% for males and ↓9-20% for females) and 5000 ppm (↓10-26% for males and ↓16-37% for females). In 500 ppm females there was a decrease in body weight gains from weeks 0-1 (↓18%) and from weeks 0-26 (↓7%). See table 4.

Table 4: Intergroup comparison of bodyweight gain – carcinogenicity and toxicity studies combined (g) - selected weeks

Week	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
0-1	39 ± 8.0	43* ± 4.7 (↑10%)	43* ± 5.9 (↑10%)	38 ± 4.6	29** ± 10.4 (↓26%)	25 ± 4.5	24 ± 5.2	21** ± 4.9 (↓18%)	20** ± 5.0 (↓20%)	16**± 4.2 (↓37%)
0-4	132 ± 17.9	145** ± 15.1 (↑10%)	131 ± 18.5	115** ± 16.1 (↓14%)	98** ± 17.5 (↓26%)	69 ± 10.1	72 ± 13.6	64 ± 10.2	59**± 10.7 (↓14%)	53** ± 7.7 (↓23%)
0-13	234 ± 33.4	247 ± 32.2	228 ± 32.3	210** ± 33.0 (↓10%)	199** ± 29.0 (↓15%)	115 ± 14.8	116 ± 18.8	109 ± 13.8	105** ± 15.6 (↓9%)	97** ± 12.2 (↓16%)
0-26	297 ± 37.1	310 ± 41.4	285± 39.7	265** ± 37.3 (↓11%)	258** ± 37.2 (↓13%)	140 ± 16.8	136 ± 20.3	130** ± 17.8 (↓7%)	125**± 16.9 (↓11%)	114** ± 12.6 (↓18%)
0-52	385 ± 48.2	401± 57.8	375 ± 54.5	352**± 53.1 (↓8%)	341** ± 41.7 (↓11%)	180 ± 35.1	181 ± 35.9	171 ± 38.1	164** ± 37.0 (↓9%)	139** ± 19.3 (↓23%)
0-78	439 ± 66.1	462 ± 79.7	437 ± 66.7	403** ± 64.5 (↓8%)	394** ± 51.9 (↓10%)	247 ± 48.0	253 ± 50.9	235 ± 57.1	219** ± 50.7 (↓11%)	175** ± 30.6 (↓29%)
0-104	468 ± 74.2	493 ± 88.1	457 ± 73.3	422** ± 63.8 (↓10%)	414** ± 57.0 (↓11%)	287 ± 54.0	285 ± 65.4	280 ± 66.0	244** ± 55.0 (↓15%)	201** ± 41.5 (↓30%)

Data were taken from pages 98-107 of the study report

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Food consumption, utilization and compound intake: Decreases in food consumption and food utilization were seen in 2500 and 5000 ppm males and females, with decreases in food utilization observed sporadically in 500 ppm females. See tables 5, 6, and 7.

Table 5: Intergroup comparison of food consumption (g/animal/day) – carcinogenicity and toxicity studies combined (g) - selected weeks

Week	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
1	20.8 ± 1.6	22.0* ± 1.1 (↑6%)	21.6 ± 1.1	20.6 ± 1.3	19.9 ± 1.6	17.1 ± 0.7	17.5 ± 1.8	16.8± 1.2	16.6 ± 1.4	16.4 ± 2.3
3	24.0 ± 1.6	24.1 ± 1.4	22.9 ± 2.7	19.8**± 4.3 (↓18%)	17.7** ± 4.2 (↓26%)	16.5± 2.6	17.7 ± 0.8	15.8 ± 2.5	14.6* ± 2.5 (↓12%)	15.2 ± 1.9
12	20.9 ± 1.4	21.4 ± 1.4	21.2 ± 1.0	21.1 ± 1.2	20.1 ± 1.4	16.5 ± 0.8	17.1 ± 1.0	17.4* ± 1.0	16.7 ± 1.1	16.2± 1.0
16	22.2 ± 1.1	22.8 ± 1.4	23.1 ± 1.2	22.3 ± 0.9	21.4 ± 1.0	18.2 ± 1.0	17.8 ± 1.1	18.8 ± 1.1	18.0 ± 1.0	17.7 ± 1.1
32	22.4 ± 1.3	22.4 ± 1.4	21.8 ± 1.0	21.1**± 0.9 (↓6%)	20.7** ± 1.0 (↓8%)	18.0 ± 1.0	17.7 ± 0.8	18.3 ± 1.3	17.7 ± 1.0	16.6** ± 1.0 (↓8%)

60	22.5 ± 1.0	22.1 ± 1.1	20.9** ± 1.1 (↓7%)	20.8** ± 1.2 (↓8%)	20.1** ± 1.1 (↓11%)	18.7 ± 1.5	19.3 ± 1.4	18.9 ± 1.4	18.4 ± 1.6	17.4 ± 1.3
80	22.3 ± 2.0	22.2 ± 1.0	21.4 ± 1.0	21.0* ± 1.4	21.0 ± 1.8	19.1 ± 1.5	19.7 ± 1.3	19.3 ± 1.8	18.3 ± 1.1	17.5* ± 1.5 (↓8%)
104	21.3 ± 2.7	22.6 ± 1.1	22.1 ± 2.0	22.0 ± 1.9	20.1 ± 1.3	18.6 ± 2.1	19.3 ± 1.7	19.1 ± 1.0	17.3 ± 1.3	17.1± 2.4

Data were taken from pages 108-113 of the study report

* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Table 6: Intergroup comparison of food utilization (weight gained (g)/100g food) – carcinogenicity and toxicity studies combined (g) - selected weeks

Week	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
1-4	21.4 ± 0.9	22.2 ± 0.9	21.3 ± 1.1	19.4**± 1.1 (↓9%)	-	14.5 ± 1.2	14.5 ± 1.4	13.4* ± 0.9 (↓8%)	12.7** ± 1.1 (↓12%)	11.6**± 1.1 (↓20%)
5-8	10.2 ± 0.9	9.8 ± 0.8	9.6 ± 0.8	9.7 ± 1.2	10.3± 1.9	5.4 ± 1.3	5.6 ± 0.5	5.4 ± 0.5	5.9 ± 1.1	5.4 ± 1.4
9-13	4.7 ± 0.8	4.8 ± 1.0	4.7 ± 0.7	4.7 ± 0.7	5.6 ± 1.2	3.2 ± 1.0	2.6 ± 0.4	2.6 ± 0.4	2.8 ± 0.4	3.2 ± 0.7
1-13	11.5 ± 0.6	11.8 ± 0.7	11.3 ± 0.4	10.7** ± 0.6 ± (↓7%)	-	7.3 ± 0.6	7.2 ± 0.6	6.7** ± 0.5 (↓8%)	6.7* ± 0.5 (↓8%)	6.4** ± 0.6 (↓12%)

Data were taken from pages 114-115 of the study report

- Food utilization not calculated due to incomplete food consumption data

* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Dose rates (based on nominal dietary levels of bicyclopyrone) were calculated in terms of mg bicyclopyrone/kg body weight. Mean values are shown below:

Table 7: Mean dose received (mg/kg bw/day)

	Carcinogenicity study				Chronic toxicity study			
bicyclopyrone (ppm)	5	500	2500	5000	5	500	2500	5000
Males	0.28	28.4	141	280	0.32	32.6	166	335
Females	0.35	35.8	178	368	0.39	41.6	204	404

Data were taken from pages 60-83 of the study report

Water consumption: There were no observable differences between treated and control groups.

Ophthalmoscopic examination: Clinical observations of severe ocular findings were noted throughout treatment from approximately week 4, in which most animals treated at 500 ppm and above were noted with opaque eyes and/or corneal damage (neovascularisation). Additionally, dull corneal surface was seen in 8, 6 and 4% of females at 500, 2500 and 5000 ppm respectively. See table 8.

Table 8: Intergroup comparison of selected organ weights (absolute and covariance analysis) – chronic toxicity study

Organ	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Eye (s) Opaque (No. Days from – to)	1 (403-737)	3 (387-737)	51 (24-737)	51 (23-737)	51 (23-737)	0	1 (640-710)	52 (24-738)	52 (24-738)	50 (24-738)
Corneal Damage (No. Days from – to)	0	1 (352-359)	51 (24-737)	51 (24-737)	51 (24-737)	0	0	51 (24-738)	52 (24-738)	50 (24-738)

Data were taken from page 43 of the study report

Functional observation battery:

Detailed clinical observations: An absent pupillary reflex was noted in 2/12, 4/12 and 2/12 males and in 4/12, 4/12 and 5/11 females treated at 500, 2500 or 5000 ppm respectively.

Motor activity: There were no treatment-related effects.

Quantitative functional observations: There were no treatment-related effects. Lower hind grip strength was observed in males treated at 2500 and 5000 ppm bicyclopyrone during the functional observational battery assessments. In the absence of any other differences in measured parameters in the functional observational battery or any effect on pathology of the central or peripheral nervous system those isolated findings are considered not to be of toxicological significance.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment across the sampling times.

Blood clinical chemistry: Blood chemistry analysis throughout treatment indicated a consistent increase in both blood glucose and phosphate levels in males and females treated at 2500 ppm and above. A decrease in creatine phosphokinase levels was noted in males and females at 2500 or 5000 ppm at various sampling times throughout the study. In the absence of any clear consistent pattern of change or any evidence of adverse histopathological findings in key organs these changes are considered not to be of toxicological significance.

Coagulation: There were no differences in coagulation parameters which were considered to be related to treatment.

Urinalysis: At termination, an increase in specific gravity coupled with an increase in urinary protein, achieving statistical significance at 500 ppm and above in treated males. Statistically significant increases in urinary ketones at 500 ppm and above were noted in both sexes. Phenylketones are excreted as a consequence of the inhibition of 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) by bicyclopyrone. The increase in urinary ketones in this study is considered to reflect an increase in phenylketones, metabolic products of tyrosine catabolism. Blood pigments were also increased at 2500 and 5000 ppm treated animals.

Sacrifice and pathology:

Organ weights:

Chronic toxicity study: Following adjustment for body weight, a statistical increase in mean covariant kidney weights compared to concurrent control, was noted in males treated at 500, 2500 or 5000 ppm (↑19, 16, 20%). There was no evidence of a dose-response. Mean brain weights (absolute and covariant) were statistically significantly reduced compared to concurrent controls in males treated with 500 ppm and above. There was no evidence of a dose-response. In females, mean heart and uterine weights (absolute and covariant) were statistically significantly decreased at 5000 ppm compared to concurrent control. See table 9.

Table 9: Intergroup comparison of selected organ weights (absolute and covariance analysis) – chronic toxicity study

Organ	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Kidney (Abs.)	2.60 ± 0.20	2.56 ± 0.21	2.84 ± 0.29	2.82 ± 0.28	2.82 ± 0.24	1.91 ± 0.26	1.89 ± 0.30	1.94 ± 0.23	1.93 ± 0.19	1.77 ± 0.15
Kidney (Adjs.)	2.45 ± 0.05	2.51 ± 0.05	2.91** ± 0.05 (↑19%)	2.83** ± 0.05 (↑16%)	2.93**± 0.05 (↑20%)	1.88 ± 0.06	1.88 ± 0.05	1.91 ± 0.05	1.93 ± 0.05	1.84 ± 0.06

Data were taken from pages 187-192 of the study report

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Carcinogenicity study: Terminal group mean body weights were reduced in comparison to respective controls for both males and females treated with 2500 or 5000 ppm bicyclopyrone. Statistically significantly higher mean covariate liver and kidney weights in males and lower brain and heart weights in both sexes, compared to concurrent controls, were noted and are detailed below. There was no clear evidence of a dose-response. Following adjustment for body weight, a statistically significant ($p<0.01$) increase in mean covariate kidney weight was seen in males treated with 500, 2500 and 5000 ppm. Mean covariant liver weights were statistically significantly increased ($p<0.01$) in males treated at 500, 2500 and 5000 ppm. Mean covariant brain weights were statistically significantly reduced ($p<0.01$) in males at 500 and 5000 ppm and in females at 500, 2500 and 5000 ppm ($p<0.01$). Mean covariant heart weights were statistically significantly decreased in males at 2500 or 5000 ppm ($p<0.05$) and females at 5000 ppm ($p<0.01$). See tables 10 and 11.

Table 10: Intergroup comparison of selected absolute organ weights – carcinogenicity study

Organ	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Kidney	3.02 ± 0.31	3.27 ± 0.78	3.40** ± 0.58 (↑13%)	3.30 ± 0.44	3.24 ± 0.37	2.33 ± 0.31	2.45 ± 0.32	2.39 ± 0.27	2.27 ± 0.28	2.14* ± 0.27 (↓8%)
Heart	1.50 ± 0.29	1.51 ± 0.17	1.42 ± 0.17	1.35** ± 0.16 (↓10%)	1.33** ± 0.16 (↓11%)	1.13 ± 0.14	1.15 ± 0.13	1.11 ± 0.13	1.04* ± 0.09 (↓8%)	0.98** ± 0.10 (↓13%)
Liver	16.76 ± 2.53	18.29* ± 2.66	18.37* ± 2.99	17.66 ± 2.43	17.40 ± 2.49	12.19 ± 2.29	12.59 ± 2.49	12.29 ± 1.80	11.80 ± 2.10	10.90* ± 1.70

		(↑9%)	(↑10%)							(↓11%)
Brain	2.26 ± 0.10	2.18 ± 0.09	2.10* ± 0.08 (↓7%)	2.11* ± 0.08 (↓7%)	2.08** ± 0.09 (↓8%)	2.02 ± 0.07	2.02 ± 0.06	1.95** ± 0.07 (↓3%)	1.95** ± 0.07 (↓3%)	1.92** ± 0.08 (↓5%)

Data were taken from pages 193-198 of the study report

* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Table 11: Intergroup comparison of selected organ weights (covariant analysis) – carcinogenicity study

Organ	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Kidney	2.95 ± 0.07	3.12 ± 0.07	3.40** ± 0.07 (↑15%)	3.39** ± 0.07 (↑15%)	3.53** ± 0.07 (↑20%)	2.24 ± 0.04	2.39* ± 0.04 (↑7%)	2.32 ± 0.04	2.32 ± 0.04	2.31 ± 0.04
Heart	1.48 ± 0.03	1.46 ± 0.03	1.42 ± 0.03	1.38* ± 0.03 (↓7%)	1.36* ± 0.03 (↓8%)	1.11 ± 0.02	1.13 ± 0.02	1.09 ± 0.02	1.06 ± 0.02	1.04* ± 0.02 (↓6%)
Liver	16.34 ± 0.32	17.29 ± 0.32	18.41** ± 0.31 (↑13%)	18.28** ± 0.30 (↑12%)	18.12** ± 0.31 (↑11%)	11.53 ± 0.27	12.09 ± 0.26	11.78 ± 0.26	12.19 ± 0.27	12.22 ± 0.28
Brain	2.15 ± 0.01	2.17 ± 0.01	2.10* ± 0.01 (↓2%)	2.11 ± 0.01	2.09*± 0.01 (↓3%)	2.01 ± 0.01	2.02 ± 0.01	1.94** ± 0.01 (↓3%)	1.95** ± 0.01 (↓3%)	1.93** ± 0.01 (↓4%)

Data were taken from pages 193-198 of the study report

* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Macroscopic findings: Opaque eyes were recorded in a large number of 500, 2500, and 5000 ppm animals from both the 52 and 104 week studies (see table 8 above). In addition, abnormal shape of the eyes (due to corneal damage) was recorded in 500, 2500 and 5000 ppm animals at 104 weeks. All other necropsy findings were considered background findings associated with this age and strain of rat, on a 52 week toxicity or 104 week carcinogenicity study.

Microscopic findings

Non-neoplastic:

Toxicity study: Minimal to marked keratitis and regenerative hyperplasia of the cornea of the eye were present in all but a few animals given bicyclopyrone at 500 ppm and above (≥83% for keratitis and ≥58% for the regenerative hyperplasia of the cornea). The findings correlated with necropsy findings of opaque eyes and ophthalmology findings of corneal damage. The severity of this lesion was greater in males than in females. The increase in the incidences of keratitis ($p<0.001$) and regenerative hyperplasia of the corneal epithelium ($p<0.001$ for 500 and 5000 ppm and $p<0.01$ for 2500 ppm) were statistically significant when compared to controls.

There was a higher incidence of hypertrophy of follicular cells of the thyroid gland in animals treated at 500 ppm and above (≥66% for males and ≥42% for females). The increase in follicular cell hypertrophy of the thyroid was statistically significant in males treated at 500, 2500 ppm ($p<0.001$) and 5000 ppm ($p<0.01$), and in females treated at 2500 and 5000 ppm ($p<0.05$). There was no clear dose response.

A higher recorded incidence of Harderian gland alteration of the exorbital lachrymal gland reached statistical significance in males treated at 500 ppm ($p<0.01$) and at 5000 ppm ($p<0.05$). Harderian gland alteration in male animals was also associated with minimal to moderate chronic inflammation in the affected acini of the lachrymal gland, and correlated with the necropsy findings of speckled, pale or pale focus recorded in male animals. See table 12.

Table 12: Intergroup comparison of the incidence of selected non-neoplastic microscopic findings – toxicity study (n=12 per sex/per dose)

Finding	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Eye: Keratitis	0	0	12*** (100%)	10*** (83%)	12*** (100%)	0	0	12*** (100%)	11*** (92%)	11*** (92%)
Eye: Regenerative hyperplasia, corneal	0	0	12*** (100%)	7** (58%)	12*** (100%)	0	0	11*** (92%)	7** (58%)	9*** (75%)
Thyroid: focal cell hypertrophy	0	0	9*** (75%)	10*** (83%)	8*** (66%)	0	0	0	5* (42%)	6* (52%)
Thyroid: focal follicular cell hyperplasia	4	2	2	3	4	1	1	0	4	0

Data were taken from pages 231-263 of the study report
* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$
*** Statistically significant difference from control group mean, $p<0.001$

Carcinogenicity study: Statistically significant increases in the incidence of keratitis (≥73% for both sexes) and regenerative hyperplasia (≥35% for both sexes) in the eye were present in all groups treated at 500 ppm and above ($p<0.001$). There was a statistically significant increase in the incidence of focal follicular cell hyperplasia in the thyroid gland in male animals treated at 5 ($p<0.05$), 500, 2500 ($p<0.01$) and 5000 ppm ($p<0.001$). Incidences were for 5, 500, 2500 and 5000 ppm were 19, 23, 23 and 33% compared to 4% in the control males.

The incidence of focal follicular cell hyperplasia was low (4%) in the control males in this study when compared to the historical control range. In a concurrent study, and one conducted shortly after (see Table 13), the incidence of thyroid hyperplasia in control male rats was 14 and 10%, respectively. Changes observed in the thyroid gland were consistent with a mild perturbation of thyroid function. At dietary concentrations of 500 ppm and above there was a consistent effect on the thyroid gland in male rats. A clear, but not dose related, increase in the incidence of focal cell hypertrophy was noted after 1 year of treatment. After 2 years a clear, but not dose related, increased incidence of focal follicular cell hyperplasia was evident. In contrast, in 5 ppm males, the incidence of focal follicular cell hyperplasia was highlighted as statistically significantly increased when compared to a low concurrent control value after 2 years of treatment whereas there was no evidence of an effect in 5 ppm males in the toxicity phase of the study.

It is concluded that although a treatment related effect on the thyroid at 5 ppm cannot be excluded it is not consistent with the effects noted at 500 ppm and above in that no change in

pathology was apparent after 1 year of treatment. A firm conclusion on the relationship to treatment is further complicated by a low concurrent control incidence focal follicular cell hyperplasia in the carcinogenicity phase.
There was a statistically significant increase in the incidence of acinar cell atrophy in the pancreas of male animals treated at 2500 ppm (50%, $p<0.05$) and 5000 ppm (58%, $p<0.01$).

There was a statistically significant increase in the incidence of Harderian gland alteration in males treated at 5000 ppm (63%, $p<0.05$), and inflammation/inflammatory cell infiltration in the lachrymal glands of male animals treated at 2500 (29%, $p<0.01$) and 5000 ppm (63%, $p<0.001$).

There was a statistically significant increase in the incidence of chronic progressive nephropathy of the kidneys in male animals treated at 5 (63%, $p<0.01$), 500, 2500 and 5000 ppm ($\geq$69%, $p<0.001$), while the incidence of pelvic mineralisation in the kidneys was decreased in males given 5000 ppm ($p<0.05$) and females treated at 500 ppm and above ($p<0.001$ for all 3 groups). An increase in the incidence of pelvic mineralization of the kidneys in females treated at 5 ppm ($p<0.05$) was considered incidental to exposure to bicyclopyrone.

According to the study authors, chronic progressive nephropathy is a common spontaneous age-related finding particularly in male rats. The incidence recorded in males at 5 ppm in this study was within historical control incidence at this laboratory. In addition no effect on kidney weight or urine clinical chemistry analysis was noted at 5 ppm whereas at 500 ppm and above there was a statistically significant increase in group mean covariate kidney weight compared to the concurrent control and a significant increase in urine specific gravity and urinary protein. Historical control data are presented on table 14.

There was a statistically significant decrease in the incidence of cardiomyopathy in male animals treated at 500 (31%, $p<0.05$), 2500 and 5000 ppm (19 and 15%, $p<0.001$).

Table 13: Intergroup comparison of the incidence of selected non-neoplastic microscopic findings – carcinogenicity study (n=52 per sex/dose)

Finding	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Eye: Keratitis	1	1	46*** (88%)	43*** (83%)	46*** (88%)	0	0	45*** (87%)	45*** (87%)	38*** (73%)
Eye: Regenerative hyperplasia, corneal	1	0	32*** (62%)	33*** (63%)	37*** (71%)	0	0	22*** (42%)	30*** (58%)	18*** (35%)
Thyroid: focal cell hypertrophy	0	1	2	1	1	0	0	1	2	2
Thyroid: focal follicular cell hyperplasia	2	10* (19%)	12** (23%)	12** (23%)	17*** (33%)	3	3	10* (19%)	6	2
Pancreas: acinar atrophy +/- inflammatory cell infiltration	14	9	19	26* (50%)	30** (58%)	7	6	6	6	10
Lachrymal gland: Harderian gland alteration	23	22	31	28	33* (63%)	3	4	3	6	7
Lachrymal gland: Inflammation / inflammatory cell infiltration	4	8	12	15** (29%)	21*** (63%)	1	3	1	1	1
Kidney: Chronic progressive nephropathy	17	33** (63%)	39*** (75%)	40*** (77%)	36*** (69%)	11	12	16	11	11
Kidney: Pelvic mineralisation	8	13	2	3	1* (2%)	29	42* (81%)	10*** (19%)	5*** (10%)	2*** (4%)
Heart: Cardiomyopathy	27	17	16* (31%)	10*** (19%)	8*** (15%)	7	6	6	2	3

Data were taken from pages 264-301 of the study report
* Statistically significant difference from control group mean, p<0.05
** Statistically significant difference from control group mean, p<0.01
*** Statistically significant difference from control group mean, p<0.001

Table 14: **Historical control incidence of selected non-neoplastic microscopic findings in male Crl:Han Wistar rats**

Year of study	2004	2005	2006	2007	2006	2007	2007	2009	Range %
Total number of animals examined	50	100	100	47	108	52	52	52	
Focal follicular cell hyperplasia	0	9	12	1	10	2	7	5	
% incidence	0	9	12	2	10	4	14	10	0-14
Chronic progressive nephropathy	28	28	59	22	30	17	33	17	
% incidence	56	28	59	47	28	33	63	33	28-63

Neoplastic: Squamous cell carcinoma of the cornea of the eye was recorded in 2/52 males animals from each of the groups treated at 500, 2500 and 5000 ppm (all 4%). Squamous cell papilloma of the cornea of the eye was recorded in 1/52 male animals from each of the groups

treated at 500 and 2500 ppm and in 3/52 animals treated at 5000 ppm (2, 2 and 6%). The tumors were associated with keratitis and regenerative hyperplasia of the cornea. According to the study authors, the incidence of the tumors did not reach statistical significance when analysed with the Fisher's Exact Test. See table 15.

Table 15: Intergroup comparison of the incidence of selected neoplastic microscopic findings – carcinogenicity study

Finding	Dietary Concentration of bicyclopyrone (ppm)									
	Males					Females				
	0	5	500	2500	5000	0	5	500	2500	5000
Cornea: Squamous cell carcinoma (malignant)	0	0	2 (4%)	2 (4%)	2 (4%)	0	0	0	0	0
Cornea: Squamous cell papilloma (benign)	0	0	1 (2%)	1 (2%)	3 (6%)	0	0	0	0	0

Data were taken from pages 342-664 of the study report

INVESTIGATOR'S CONCLUSIONS

Dietary administration of bicyclopyrone to rats at 0, 5, 500, 2500 and 5000 ppm, for a period of up to 104 Weeks, was associated with in-life effects (reduced body weights, food consumption and food utilisation) in rats treated at 2500 or 5000 ppm. No evidence of any differences in the survival patterns were seen for the treated groups in either sex when compared to the control animals.

Dietary treatment with bicyclopyrone was associated with the occurrence of squamous cell carcinoma and papilloma, and opacity, keratitis and regenerative hyperplasia of the cornea of the eye at dietary concentrations of 500 ppm and above.

Treatment with bicyclopyrone was associated with non neoplastic findings in the thyroid gland, kidneys (with organ weight increases and urine clinical chemistry changes), exocrine pancreas, heart (with associated organ weight differences) and the lachrymal gland.

The NOEL for findings in the exocrine pancreas, the heart and the lachrymal gland was 500 ppm, while the NOAEL for chronic progressive nephropathy in the kidney and focal follicular cell hyperplasia of the thyroid gland in males was considered to be 5 ppm.

Therefore, a clear No Observed Adverse Effect Level (NOAEL) for this study was considered to be 5 ppm for both males and females equating to 0.28 mg/kg/day NOA449280 in males and 0.35 mg/kg/day in females.

REVIEWER COMMENTS

The purpose of this study was to determine the toxicities in rats resulting from 1-2 years of exposure to bicyclopyrone through the oral route. Five groups of 52 male and 52 female Han Wistar rats were assigned to the Carcinogenicity study and dosed with diets containing 0, 5, 500, 2500 or 5000 ppm bicyclopyrone for at least 104 consecutive weeks. In addition, a chronic toxicity study comprising a further 5 groups of 12 males and 12 females was included and dosed in an identical fashion for a period of 52 consecutive weeks. The equivalent doses for the

carcinogenicity phase of the study were 0, 0.28/0.35, 28.4/35.8, 141/178 and 280/368 mg/kg/day (M/F). The equivalent doses for the chronic toxicity phase of the study were 0, 0.32/0.39, 32.6/41.6, 166/204 and 335/404 mg/kg/day (M/F). Under the conditions of the study, the adverse effects of this study are as follow:
At 5 ppm bicyclopyrone, there was a 2-6% increase in the incidence of opaque eyes and corneal damage in both sexes compared to the control group (0-2%). At 104 weeks in males, there was an increased incidence of thyroid follicular hyperplasia in males (19%) compared to the control group (4%). There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (63%) compared to the control group (33%).

At 500 ppm bicyclopyrone, there was a significant increase in the incidence of opaque eyes and corneal damage in both sexes (98-100%) compared to controls (0-2%). There was an increase in the incidence of eye keratitis (88-100% for males and 87-100% for females) and the regenerative corneal hyperplasia (88-100% for males and 42-92% for females) from 52 weeks to 104 weeks compared to the control group (2%). In males, there was an increased incidence of thyroid follicular hypertrophy (75%) at 52 weeks compared to the control group (0%). At 104 weeks in males, there was an increased incidence of thyroid follicular hyperplasia (23%) compared to the control group (19%). This effect occurred in females as well but there was no dose response. There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (75%) compared to the control group (33%). In males, there was an increased incidence of squamous cell carcinoma and papilloma (4% and 2%) compared to the control group (0%).

At 2500 ppm bicyclopyrone, there was a significant increase in the incidence of opaque eyes and corneal damage in both sexes compared to controls (98-100%) compared to the control group (0-2%). Decreases in absolute body weights for females were transiently statistically significant through the study (↓5-10%). Relative to the control group, there was a minor decrease in the absolute brain weights of males and females (↓3-7%), and heart weights of females (↓7%). There was an increase in the incidence of eye keratitis (83% for males and 87-92% for females) and regenerative corneal hyperplasia (58-63% for males and 58% for females) from 52 weeks to 104 weeks compared to the control group (2%). In males, there was an increased incidence of thyroid follicular hypertrophy (83%) at 52 weeks compared to the control group (0%). At 104 weeks in males, there was an increased incidence of thyroid follicular hyperplasia (23%) compared to the control group (4%). There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (77%) compared to the control group (33%). There was a statistically significant increase in the incidence of acinar cell atrophy in the pancreas of male animals (50%) compared to the control group (27%). In males, there was an increased incidence of squamous cell carcinoma and papilloma (4% and 2%) compared to the control group (0%).

At 5000 ppm bicyclopyrone, there was a significant increase in the incidence of opaque eyes and corneal damage in both sexes (98-100%) compared to the control group (0-2%). Relative to the control group, in both sexes there were significantly lower body weights (↓5-16% for males and ↓5-20% for females). There were also minor changes in food consumption and utilization. There was an increase in the incidence of eye keratitis (88-100% for males and 73-92% for females) and the regenerative corneal hyperplasia (71-100% for males and 35-75% for females) from 52 weeks to 104 weeks compared to the control group (2%). In males, there was an increased incidence of thyroid follicular hypertrophy (66%) at 52 weeks compared to the control group (0%). At 104 weeks in males, there was an increased

incidence of thyroid follicular hyperplasia (33%) compared to the control group (4%). There was also an increase in the incidence of chronic progressive nephropathy in the kidneys of males (69%) at 104 weeks compared to the control group (33%). There was a statistically significant increase in the incidence of acinar cell atrophy in the pancreas of male animals (58%) compared to the control group (27%). In males, there was an increased incidence of squamous cell carcinoma and papilloma (4% and 6%) compared to the control group (0%).

Based upon the effects in this study, the LOAEL for systemic toxicity is 5 ppm (0.28/0.35 mg/kg/day [M/F]) based on a dose dependent increase in the incidence of opaque eyes and corneal damage in both sexes compared to controls, an increased incidence of thyroid follicular hyperplasia in males, and an increased incidence of chronic progressive nephropathy in the kidneys of males. The NOAEL was not established.

The corneal tumors seen in males rats are associated with and likely attributable to significant damage to and regenerative hyperplasia of the cornea seen during the course of the carcinogenicity study with bicyclopyrone at concentrations of 500 ppm and above. The identified mode of action of HPPD inhibiting herbicides results in significantly elevated plasma tyrosine in rats, particularly males. EPA's Cancer Assessment Review Committee determined that in male rats, there was a dose-dependent increase in corneal tumors which were considered treatment related (Rowland et al, September 10, 2014, TXR #0057011). The doses tested were considered to be adequate and not excessive, for assessing carcinogenicity in both sexes. This was based upon increases in corneal opacity, decreased absolute body weights in both sexes at the high dose, and an increased incidence of regenerative corneal hyperplasia in both sexes.

This study is classified as totally reliable **(acceptable/guideline)** as a combined chronic/carcinogenicity study in rats (OPPTS 870.4300; OECD 451). EPA, PMRA (Canada), and APVMA/OCS (Australia) agree regarding the classification of this study but not the regulatory decision. APVMA believes that the chronic progressive nephropathy and thyroid follicular hyperplasia are both within the historical control range and are thus not adverse. It is their conclusion that the NOAEL should be 5 ppm, as all three effects relied on for LOAEL selection are not considered toxicologically adverse. APVMA further disagrees with EPA in thinking that there are no lesions within the study worthy of being considered carcinogenic.

(Robertson B and Perry C, 2012)

EPA Reviewer: Anwar Dunbar, Ph.D. **Signature:** _____
Risk Assessment Branch I, Health Effects Division (7509P) Date: _____
EPA Reviewer: Greg Akerman, Ph.D. **Signature:** _____
Risk Assessment Branch I, Health Effects Division (7509P) Date: _____

TXR#: 0057111

DATA EVALUATION RECORD

PC CODE: 018986 **DP BARCODE:** D425155

STUDY TYPE: Carcinogenicity – Mouse (feeding)
OECD 451 (2009): OPPTS 870.4200 (1998): 88/302/EEC B.32 (2001): JMAFF 12 Nohsan No. 8147 (2000)

TEST MATERIAL (PURITY): NOA449280 (purity 94.5% w/w)

SYNONYMS: Bicyclo[3.2.1]oct-3-en-2-one,4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-(trifluoromethyl)-3-pyridinyl]carbonyl]- ; 4-hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-(trifluoromethyl)-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one; bicyclopyrone, SYN449280

CITATION: Robertson B, 2012. NOA449280: 80 week mouse dietary carcinogenicity study. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 30195, 17 August 2012. Unpublished. (Syngenta File No. NOA449280_11243). MRID 47841987

SPONSOR: Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, United Kingdom.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In a carcinogenicity study in mice (MRID #47841987), bicyclopyrone (NOA449280, purity 94.5% w/w) was administered to groups of 50 male and 50 female CD-1 mice in the diet at dose levels of 0, 70, 1700 and 7000 ppm (equivalent to 0, 8.7 / 9.2, 233 / 242, 940 / 1027 mg/kg bw/day for males / females respectively) for a period of at least 80 weeks.

Animals were monitored regularly for viability and for signs of ill health or reaction to treatment. Body weights and food consumption were measured and recorded at predetermined intervals from pretrial up until the completion of treatment. At week 80, prior to terminal kill, blood samples were collected from all surviving animals for haematological analysis. Blood films were made from all surviving animals during week 53/54 and at week 80; however, blood cell morphology was not performed as no treatment related effects were seen on white cell haematological parameters at termination. All surviving animals were terminated and subjected to a detailed necropsy examination after completion of treatment. Tissues from all animals were subject to a comprehensive histological evaluation.

There were no treatment related clinical observations and no effects on mortality at any dose.

At all doses, there were changes at 70 and 1700 ppm, such as increased absolute and adjusted liver weights which were considered adaptive.

At 7000 ppm bicyclopyrone, there was a statistically significant decrease in the absolute body weights in females (↓9-12%).

At the highest dose tested, there was an increased incidence of lung adenomas which were determined to not be treatment related and thus not toxicologically significant.

Based upon the effects in this study, the LOAEL is 7000 ppm (1027 mg/kg/day [F]) based upon decreased absolute body weights in females. The NOAEL is 1700 ppm (242 mg/kg/day [F]). The NOAEL for males is 7000 ppm (940 mg/kg/day). The LOAEL for males was not established.

This study is classified as totally reliable (**acceptable/guideline**) and satisfies the guideline requirements (OPPTS 870.4200) for an oncogenicity study in the mice.

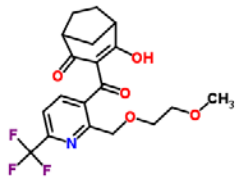
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. Deviations occurring during the study were minor and did not compromise the quality of the study.

MATERIALS AND METHODS

Materials:

Test Material:	Bicyclopyrone (NOA449280)
Description:	Technical material, brown/beige powder
Lot/Batch number:	SEZ3AP006/MILLED
Purity:	94.5% a.i
CAS#:	352010-68-5
Stability of test compound:	Reanalysis March 2011

Structure:



Vehicle and/or positive control: The test substance was administered via Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) (Special Diets Services Limited, 1 Stepfield, Witham, Essex, UK).

Test Animals:

Species	Mouse
Strain	CD-1 mice (Crl:CD-1(ICR))
Age/weight at dosing	Approximately 6 weeks / 26.1-40.9 g (males), 22.6-32.2 g (females)
Source	Charles River UK Limited, Margate, Kent, UK
Housing	Male animals housed individually and females up to 3 per cage in suspended polycarbonate cages with stainless steel grid tops.
Acclimatisation period	Approximately 2 weeks
Diet	Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) (Special Diets Services Limited, 1 Stepfield, Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 12-26°C (intended range 19-23°C) Humidity: 29.58-80.33% (intended range 40-70%) Air changes: Minimum of 15/hour Photoperiod: 12 hours light / 12 hours dark

In-life dates: Start: 04 October 2007 End: 09 September 2009

Study Design and Methods: In a carcinogenicity study NOA449280 (purity 94.5% w/w) was administered to groups of 50 male and 50 female CD-1 mice in the diet at dose levels of 0, 70, 1700 and 7000 ppm (equivalent to 0 / 0, 8.7 / 9.2, 233 / 242, 940 / 1027 mg/kg bw/day for males / females respectively) for a period of at least 80 weeks.

Animal assignment: On arrival from the suppliers, the animals were allocated to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Each month from the commencement of pretrial, each column of cages on a rack were moved one position along the racks assigned to that sex, with the end column returning to the start of the first rack, to minimise environmental effects. The control animals were housed on a separate rack from the treatment groups. During pretrial, group mean body weights were checked to ensure that all groups had a similar body weight for each sex.

Table 1: Study design

Test group	Dietary concentration (ppm)	# male	# female
Control	0	1-50	201-250
Low	70	51-100	251-300
Mid	1700	101-150	301-350
High	7000	151-200	351-400

Table was taken from pages 21 of the study report

Diet preparation and analysis: Experimental diets were prepared by direct admixture of test item to a required amount of untreated diet and blended for 20 minutes in a diet mixer. Blank diet (without the test substance under investigation) was prepared for Control animals. Diet formulations were prepared and dispensed once every 2 weeks.

Prior to study commencement, stability data were generated by Charles River, Edinburgh for 15 days for experimental diets stored at -20ºC in the dark, in the concentration range of 2.5-7000 ppm. During the study, triplicate samples (3 x 50 g) were taken from each experimental diet (including control) at approximately 3 monthly intervals, immediately after preparation, and analysed for achieved concentration and homogeneity.

Concentration analysis results:
Analysed concentrations of test item in experimental diets were found to be within ±4.7% (Week 1), ±2.1% (Week 13), ±3.9% (Week 26), ±1.3% (Week 39), ±0.7% (Week 52), ±3.2% (Week 65) and ±0.6% (Week 79) of the theoretical concentrations.

Homogeneity results:
The coefficient of variance was low (5.5% or below) indicating satisfactory homogeneity of diet formulations.

Stability results:
Stability for the range 2.5-7000 ppm was satisfactory for 15 days when stored at ambient temperature and protected from light, or when frozen at -20°C.

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable, provided that the cited stability study did indicate that the test compound was stable under conditions of the study.

Observations: All animals were checked early morning and as late as possible each day for signs of viability. Once each week all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Body weight: Body weights were recorded once weekly during pretrial up until week 14 of treatment, and approximately once every 2 weeks from week 15 up until the end of treatment.

Food consumption, utilization and test substance intake: The quantity of food consumed by each cage of animals was measured and recorded once weekly during pretrial up until week 14 of treatment and once every 4 weeks from week 16 up until the end of treatment.

Food utilization was calculated for weeks 1-4, 5-8, 9-13 and 1-13 according to the following formula:

(Cage mean weight gain x 100) / cage total food consumption

The amount of test item ingested was calculated at regular intervals during treatment using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{Nominal Concentration (ppm)} \times \text{Food Consumption (g/day)}}{\text{Mid-point Body Weight (g)}}$$

Water consumption: Water consumption was qualitatively monitored by visual inspection of water bottles on a weekly basis throughout the study.

Haematology: Blood was collected from all surviving animals via the orbital sinus under isoflurane anaesthesia and transferred into tubes containing EDTA prior to the terminal kill. The animals were not deprived of food overnight prior to sampling. The following parameters were examined:

total white cell count differential white cell count

A blood film smear was made from all EDTA haematology samples and stained for possible examination from all surviving animals at week 53/54 and week 80 scheduled euthanasia and

femoral bone marrow smears were taken at necropsy and stored for possible evaluation. Neither the blood nor bone marrow smears were examined as haematological findings indicated that evaluation would not yield any further information.

Investigations *post mortem:* After at least 80 weeks of treatment all surviving animals were killed in random order by exposure to carbon dioxide and had their terminal body weight recorded followed by exsanguination.

Macroscopic examination: All animals were examined *post mortem*. The necropsy consisted of a complete internal and external examination which included body orifices (ears, nostrils, mouth, anus, vulva) and cranial, thoracic and abdominal organs and tissues.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands
brain
epididymides
heart
kidneys
liver and gall bladder
ovaries
spleen
testes
uterus (with cervix)

Paired organs were weighed separately and the sum of the individual organs used for reporting purposes.

Tissue submission / microscopic examination: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative, and 4-6 µm sections all processed tissues were examined by light microscopy:

abnormal tissue (including local lymph nodes to masses)
adrenal gland
aortic arch
tongue
brain (forebrain, midbrain, cerebellum and pons)
caecum
colon
duodenum
epididymis
eyes
femur (including knee joint and bone marrow)
Harderian gland
heart
lachrymal gland
ileum
jejunum
kidney
larynx
liver and gall bladder
lung
optic nerve
oesophagus
ovary
oviduct
vagina
peyer's patches
pancreas
parathyroid gland
pharynx
pituitary gland
prostate gland
rectum
salivary gland
seminal vesicle
spinal cord (cervical, midthoracic, lumbar)
skin
spleen
sternum (including bone marrow)
stomach
testis
thymus
thyroid gland

lymph node - mesenteric	trachea
mammary gland	urinary bladder
nerve - sciatic	uterus
nasal cavity	thigh muscle

Statistics: Body weight, cumulative body weight gain, food consumption, food utilization, haematology and organ weight data were analysed using a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test. Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as covariate. Analyses of variance and covariance were carried out using the MIXED procedure in SAS (9.1.3). Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Dunnett's t-test, based on the error mean square in the analysis. All statistical tests were two sided and performed at the 5% and 1% levels.

The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant.

Kaplan-Meier survival estimates were calculated separately for each sex and treatment group. Pairwise comparisons of the incidence of tumor and histological lesions was made using Fisher's Exact test (two-tailed). Histological findings with multiple severities were also analysed using the Mann-Whitney U test. Further analyses were performed using Peto's time adjusted methods.

The statistical evaluation of the tumor data was performed in SAS (v8.2) using PROC MULTTEST. Methods used for the age-adjusted analysis of fatal and non-fatal tumors were based on the IARC guidelines.

RESULTS AND DISCUSSION

Mortality: There were no statistically significant differences in mortality for either male or female treated groups in comparison to their respective controls. Additionally, the trend test was not seen to be statistically significant for any male or female treated group.

Clinical observations: There were a variety of clinical observations recorded in control and treated mice but these were either commonly seen observations in this age and strain of mouse or were seen in small numbers of animals. None of the observations seen were considered to be related to treatment./

Bodyweight and weight gain: Absolute body weight and body weight gain data are presented in tables 2 and 3. Males and females treated at 7000 ppm showed slight decreases in absolute body weight (↓8-9% and ↓9-12%, respectively) and body weight change (17-29 and 23-55%, respectively) when compared to their control, although males treated at 7000 ppm had a statistically significantly lower initial body weight. Statistically significant differences were noted at various timepoints and the slight decreases were consistent throughout the treatment period.

Males treated at 1700 ppm showed a statistically significantly lower (↓4%) initial body weight compared to their control. However, no consistent statistically significant difference was noted after the first few weeks of the study.

Females treated at 1700 and 7000 ppm showed isolated statistically significantly higher body weight gains Weeks 0-2 (↑40% and ↑50%), and females treated at 70 ppm were noted to have an isolated statistically significantly lower body weight change during Weeks 0-3. These isolated differences were not related to treatment.

Table 2: Mean intergroup comparison of absolute bodyweights (g) by week

	Dietary Concentration of bicyclopyrone (ppm)							
	Males				Females			
Week*	**0**	**70**	**1700**	**7000**	**0**	**70**	**1700**	**7000**
Pre-Test (Week 0)	35.3 ± 2.4	35.0 ± 2.8	33.9* ± 2.9 (↓4%)	33.9* ± 2.7 (↓4%)	26.9 ± 1.8	27.0 ± 1.6	26.9 ± 1.8	26.5 ± 2.0
Week 2	38.2 ± 2.8	38.3 ± 3.3	37.5 ± 3.6	37.2 ± 3.0	28.8 ± 2.3	28.6 ± 2.1	29.6 ± 2.3	29.5 ± 2.4
Week 20	54.7 ± 6.1	54.2 ± 6.4	51.2* ± 6.8 (↓6%)	49.9** ± 5.9 (↓9%)	42.8 ± 6.2	42.9 ± 8.2	43.7 ± 8.0	39.0* ± 5.3 (↓9%)
Week 40	58.7 ± 7.0	59.1 ± 7.2	56.2 ± 7.6	54.2** ± 6.8 (↓8%)	49.9 ± 8.1	52.0 ± 10.9	50.2 ± 10.2	44.1** ± 7.0 (↓12%)
Week 80	61.9 ± 7.7	62.8 ± 8.1	58.4 ± 7.5	57.0* ± 8.4 (↓8%)	56.0 ± 8.7	58.5 ± 12.6	54.2 ± 13.0	51.4 ± 8.8

Data were taken from pages 74-81 of the study report

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Table 3: Intergroup comparison of bodyweight gain (g) - selected timepoints

	Dietary Concentration of bicyclopyrone (ppm)							
	Males				Females			
weeks	**0**	**70**	**1700**	**7000**	**0**	**70**	**1700**	**7000**
0-1	2.1 ± 0.7	2.3 ± 0.8	1.5* ± 0.8 (↓29%)	1.5* ± 0.8 (↓29%)	1.2 ± 1.0	0.6 ± 1.1	1.4 ± 1.5	1.1 ± 1.4
0-2	2.9 ± 1.2	3.2 ± 1.2	3.6** ± 1.5 (↑24%)	3.4 ± 1.1	2.0 ± 1.4	1.6 ± 1.5	2.8* ± 1.5 (↑40%)	3.1** ± 1.4 (↑55%)
0-4	5.9 ± 1.9	6.4 ± 2.1	5.9 ± 2.1	4.9* ± 1.7 (↓17%)	4.1 ± 1.9	4.2 ± 1.8	4.3 ± 1.9	3.8 ± 2.0
0-8	10.7 ± 3.1	10.9 ± 3.4	10.1 ± 3.5	8.7** ± 2.4 (↓19%)	7.7 ± 3.1	8.2 ± 3.8	7.6 ± 3.5	7.2 ± 3.4
0-26	22.5 ± 5.7	22.2 ± 5.5	19.8 ± 6.4	17.9** ± 5.3 (↓20%)	19.6 ± 6.6	21.5 ± 8.6	18.8 ± 8.3	14.0** ± 5.0 (↓29%)
0-52	26.4 ± 6.9	27.0 ± 6.7	25.3 ± 7.6	23.0* ± 6.5 (↓13%)	27.9 ± 8.5	29.7 ± 10.5	27.3 ± 10.4	21.6** ± 7.7 (↓23%)
0-80	26.5 ± 7.2	27.9 ± 6.9	24.7 ± 7.1	23.3 ± 7.7	29.4 ± 8.2	31.4 ± 12.3	27.3 ± 12.3	24.9 ± 8.4

Data were taken from pages 82-89 of the study report

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Food consumption, utilization and compound intake: Males treated at 1700 and 7000 ppm had statistically significantly lower food consumption than controls during pretrial, thereafter

there was no consistent pattern and food consumption values were both lower and higher than control values. There was no effect on food consumption in males at 70 ppm or females at any dose level.

Food utilization data are presented in table 4. Food was less efficient in males treated at 7000 ppm from Weeks 1-4 and 5-8 and this was reflected in the overall value for Weeks 1-13 (↓17%, ↓22% and ↓12%). Food utilization for males treated at 1700 ppm was less efficient than control for the period 5-8 weeks only (↓19%). Females treated at 7000 ppm showed a slightly decreased food utilization profile throughout Weeks 1-13, with statistical significance being achieved for Weeks 9-13 (↓64%), and as an overall result for Weeks 1-13 (↓33%) . The isolated difference in food utilization for females treated at 70 ppm during Weeks 9-13 (↓44%), was considered not to be related to treatment in the absence of a similar difference in females treated at 1700 ppm.

Table 4: Intergroup comparison of food utilization (g) - selected timepoints

	Dietary Concentration of bicyclopyrone (ppm)							
	Males				Females			
weeks	**0**	**70**	**1700**	**7000**	**0**	**70**	**1700**	**7000**
1-4	3.5 ± 1.1	3.8 ± 1.1	3.5 ± 1.2	2.9** ± 1.0 (↓17%)	3.5 ± 1.0	3.3 ± 0.5	3.2 ± 1.1	2.9 ± 0.8
5-8	2.7 ± 0.9	2.4 ± 0.9	2.2** ± 1.0 (↓19%)	2.1**± 0.8 (↓22%)	2.6 ± 1.2	2.8 ± 1.0	2.1 ± 1.0	2.2 ± 0.6
9-13	1.6 ± 0.9	2.0 ± 0.8	1.5 ± 1.1	1.7 ± 1.0	2.5 ± 0.9	1.4** ± 1.1 (↓44%)	2.2 ± 0.9	0.9** ± 0.8 (↓64%)
1-13	2.5 ± 0.7	2.7 ± 0.7	2.3 ± 0.8	2.2* ± 0.6 (↓12%)	2.8 ± 0.7	2.4 ± 0.7	2.4 ± 0.7	1.9** ± 0.4 (↓33%)

Data were taken from pages 96-97 of the study report

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Dose rates (based on nominal dietary levels of bicyclopyrone) were calculated in terms of mg bicyclopyrone/ kg body weight. Mean values are shown in Table 5.

Table 5: Mean dose received (mg/kg/day)

bicyclopyrone (ppm)	70	1700	7000
Males	8.7	233	940
Females	9.2	242	1027

Data were taken from pages 35-70 of the study report

Water consumption: There were no observable differences between treated and control groups.

Haematology: There were no differences in haematology parameters which were considered to be attributable to treatment with bicyclopyrone.

Higher, although not statistically significant, mean white cell counts, were noted in males treated at 70 ppm. This reflected an abnormally high value for one animal, which was diagnosed with malignant lymphoma.

Mean white cell counts in females treated at 7000 ppm were statistically significantly higher than control. This reflected higher, although not statistically significant, values for large unclassified cells, lymphocytes and neutrophils; however, it is considered that the higher values at 7000 ppm were the result of three individual animals two with lymphoid hyperplasia, and one with malignant lymphoma).

Sacrifice and pathology:
Macroscopic findings: A small number of lesions were observed, none of which were considered to be related to treatment.

Organ weights: Liver weights, adjusted for terminal body weight, were statistically significantly increased in all male treated groups (↑25%-↑28%), where no clear dose response was apparent, and in females at 1700 and 7000 ppm (↑20%-↑31%). Changes in absolute liver weights correlated with adjusted liver weights in all cases. See table 6.

Other slight variances from the control organ weights were noted in either sex, some of which were seen to be statistically significant. However, due to a lack of corroborating data, evidence of a dose related response or consistency between sexes, it is considered that these findings were not treatment related.

Table 6: Intergroup comparison of liver weights (g)

	Dietary concentration of bicyclopyrone (ppm)							
	Males				Females			
	0	70	1700	7000	0	70	1700	7000
absolute	3.11 ± 0.74	3.90** ± 1.46 (↑25%)	3.52 ± 0.99 (↑13%)	3.76* ± 1.09 (↑21%)	2.41 ± 0.46	2.59 ± 0.56	2.79** ± 0.74 (↑16%)	2.96** ± 0.75 (↑23%)
adjusted	3.03 ± 0.16	3.79** ± 0.16 (↑25%)	3.59* ± 0.17 (↑18%)	3.89** ± 0.16 (↑28%)	2.37 ± 0.08	2.45 ± 0.08	2.85** ± 0.08 (↑20%)	3.11** ± 0.09 (↑31%)

Data were taken from pages 100-103 of the study report

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Microscopic findings: A small number of spontaneous lesions were observed, none of which was related to treatment.

Non-neoplastic: Data for non-neoplastic lesions are presented on table 7. There was a statistically significant increase in the incidence of centrilobular hypertrophy ($p<0.001$) in the liver of males receiving 7000 ppm compared to controls. This finding was noted in 36/50 males receiving 7000 ppm where all gradings were mild, compared to 0/50 in controls (72% vs. 0%).

There was a statistically significantly lower incidence of adrenal subcapsular cell hyperplasia in both sexes at 7000 ppm (generally where the grading was mild), compared with controls ($p<0.05$).

Table 7: Intergroup comparison of selected non-neoplastic histopathological findings

Finding	Dietary concentration of bicyclopyrone (ppm)							
	Males				Females			
	0	70	1700	7000	0	70	1700	7000
Liver centrilobular hypertrophy	0/50	0/50	0/50	36/50*** (72%)	0/49	0/50	0/50	0/50
Adrenal subcapsular cell hyperplasia	24/50 (48%)	19/50 (38%)	15/50 (30%)	12/49* (25%)	40/49 (82%)	35/50 (70%)	33/50* (66%)	29/50* (58%)

Data were taken from pages 127-103 of the study report
* Statistically significant difference from control group mean, p<0.05
*** Statistically significant difference from control group mean, p<0.001

Neoplastic: There were no tumors considered related to treatment with bicyclopyrone. There was no difference in the overall number of tumors, the number of tumor bearing animals or the time to onset of any tumor type.

Table 8: Intergroup comparison of bronchio-alveolar histopathological findings male mice

Finding	Dietary concentration of bicyclopyrone (ppm)			
	Males			
	0	70	1700	7000
Number of mice examined	50	50	50	50
Bronchio-alveolar carcinoma (M)	2	3	3	4
Bronchio-alveolar adenoma (B)	9 (18%)	13	13	18 (36%)
Brochio-alveolar hyperplasia				
Minimal	0	0	0	1
Mild	2	1	1	1
Total	2	1	1	2

Data were taken from page 195 of the study report

The incidence of bronchiole-alveolar adenoma in the lung was numerically higher in males receiving 7000 ppm compared to concurrent controls (Table 8). A Peto trend test revealed a statistically significant increasing trend in the incidence of lung bronchiolo-alveloar adenoma [B] in males (p=0.034). When the high dose group (group 4) was excluded from the analysis, the test for increasing trend was no longer statistically significant (p=0.24). This incidence at 7000 ppm in males was not statistically significantly different from the control by pair-wise comparison. There were no differences in female mice. The incidence of benign tumors in the male lung at the limit dose of 7000 ppm was not accompanied by any other histopathological changes in the lungs and was similar to the incidence seen in control groups from studies being conducted concurrently. See table 9.

Table 9: Historical Control for Lung Bronchiolo-Alveolar Tumors and Hyperplasia: Crl:CD-1 Mice

Study identifier	Study start	Route of administration	Number of lungs examined	Males		
				Hyperplasia	Adenoma	Carcinoma
283	Sep 2007	Dietary	50	3	12	5
613	Oct 2007	Dietary	50	0	14	1
629	Sep 2007	Dietary	50	0	13	1
575	Sep 2007	Dietary	50	0	15	4
Total			**200**	**3**	**54**	**11**
			Range	**0-6%**	**24–30%**	**2-10%**

Data were taken from page 31 of the study report

This numerically higher incidence of bronchiole-alveolar adenoma in males at 7000 ppm was considered not to be treatment related.

INVESTIGATOR'S CONCLUSIONS

Dietary administration of bicyclopyrone at 0, 70, 1700 and 7000 ppm, for a period of at least 80 weeks, was associated with decreases in body weight and body weight gain and less efficient food in males and females treated at 7000 ppm.

There were no tumours considered to be related to treatment with bicyclopyrone. There was no difference in the overall number of tumours, the number of tumour bearing animals or the time to onset of any tumour type.

The No Observed Adverse Effect Level (NOAEL) for bicyclopyrone was 1700 ppm (233 and 242 mg/kg/day) in males and females respectively.

REVIEWER COMMENTS

The purpose of this study was to evaluate the carcinogenic potentiatal of pyrifluquinazon in mice for 18 months through oral exposure. Bicyclopyrone (NOA449280, purity 94.5% w/w) was administered to groups of 50 male and 50 female CD-1 mice in the diet at dose levels of 0, 70, 1700 and 7000 ppm (equivalent to 0, 8.7 / 9.2, 233 / 242, 940 / 1027 mg/kg bw/day for males / females respectively) for a period of at least 80 weeks.

There were no treatment related clinical observations and no effects on mortality at any dose. At all doses, there were changes at 70 and 1700 ppm, such as increased absolute and adjusted liver weights which were considered adaptive.

At 7000 ppm bicyclopyrone, there was a statistically significant decrease in the absolute body weights in females (↓9-12%).

EPA's Cancer Assessment Review Committee (CARC) concluded that in male mice, there was a trend and pairwise significance at the high dose for adenomas and combined tumors. However, these tumors were determined to be "not treatment related" based on the following considerations:

- Only a marginal increase in adenomas was observed in comparison to the

concurrent control. The incidence for lung adenomas in the concurrent controls was slightly lower than the historical control range for this tumor at 80 weeks which resulted in the marginal increase of adenomas;
- There was no increases in carcinomas (*i.e.* no malignant progression);
- There were no corroborative precursor lesions (i.e. hyperplasia or other non-neoplastic lesions) observed at this dose;
- Lung tumors are a common background tumor in mice of this age; and
- Lung tumors were not seen in pesticide chemicals of this class (no structural activity relationship (SAR) support).

Regarding the adequacy of dosing, the highest dose tested was the Limit Dose (7000 ppm or 1000 mg/kg/day) in both sexes and was considered to be adequate and not excessive for assessing carcinogenicity.

Based upon the effects in this study, the LOAEL is 7000 ppm (1027 mg/kg/day [F]) based upon decreased absolute body weights in females. The NOAEL is 1700 ppm (242 mg/kg/day [F]). The NOAEL for males is 7000 ppm (940 mg/kg/day). The LOAEL for males was not established.

This study is classified as totally reliable (**acceptable/guideline**) and satisfies the guideline requirements (OPPTS 870.4200) for an oncogenicity study in the mice. EPA, PMRA (Canada), and APVMA/OCS (Australia) agree on the regulatory decision and classification for this study.

(Robertson B, 2012)

DATA EVALUATION RECORD
Pyrifluquinazon
PC Code: 555555
TXR#: 0055820
MRID#: 48306965

Study Type: 18-Month Dietary Carcinogenicity Study (mouse) (2006)
OPPTS 870.4200 / OECD 451

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Tetrahedron Incorporated
1414 Key Highway, Suite B
Baltimore, MD 21230

Principal Reviewer Isabel Jo Mandelbaum Date 7-25-11
Isabel Mandelbaum, Ph.D., DABT

Secondary Reviewer Nasrin Begum Date 7-25-11
Nasrin Begum, Ph.D.

Tetrahedron Program Manager Nasrin Begum Date 7-25-11
Nasrin Begum, Ph.D.

Quality Control Date 7-25-11
Daniel Ewald, B.S.

Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-47
EPA Reviewer//WAM: Dunbar//Brunsman/Farwell

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Anwar Y Dunbar, Ph.D. **Signature:** _____
Risk Assessment Branch 1, Health Effects Division (7509P) **Date:** _____
EPA Reviewer: Chester Rodriguez, Ph.D. **Signature:** _____
Risk Assessment Branch I, Health Effects Division (7509P) **Date:** _____

TXR#: 0055820

DATA EVALUATION RECORD

STUDY TYPE: 18-Month Dietary Carcinogenicity Study – Mouse OPPTS 870.4200; OECD 451

PC CODE: 555555 **DP BARCODE**: D387307

TEST MATERIAL (PURITY): NNI-0101 Technical (Pyrifluquinazon) (98.0% a.i.)

SYNONYMS: NNI-0101 Technical, Pyrifluquinazon, R-40598 Technical grade, 1-acetyl-3,4- dihydro-3-[(3-pyridinylmethyl) amino]-6-[1,2,2,2-tetrafluro-1-(trifluoromethyl)ethyl-2(1H)-quinazolinone

CITATION: Kuwahara, M. (2006) NNI-0101 Technical: Carcinogenicity study in mice. The Institute of Environmental Toxicology (Ibaraki, Japan). Project Identification: T-29018, IET Study No. 03-0064, September 27, 2006. MRID 48306965. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd.; 2-5, Nihonbashi 1-Chome, Chuo-ku; Tokyo 103-8236, Japan.

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 48306965), NNI-0101 (98.0% a.i., Lot No. 3FZ0013G) was administered in the diet to SPF ICR (Crj:CD-1) mice (52/sex/dose) at concentrations of 0, 60, 250, or 1000 ppm (equivalent to 0, 6.25/ 5.82, 27.1/ 25.0, and 122/ 120 mg/kg/day [M/F]) for 18 months (78 weeks). All surviving animals were sacrificed at the study termination.

There were no treatment related effects at the 60 ppm concentration.

At the 250 ppm concentration, there was a persistent depression in mean body weight in males from Week 8 to 56 (↓5-8%). Slight increases in incidences of follicular cell hypertrophy of the thyroid were seen in some males. There were small incidences of tactile hair loss in males. There was also a significant increase in incidences of endometrial hyperplasia of the uterine horn in females (52% vs. 44% in the control). Finally, there was a significant increase in incidence of subcapsular cell hyperplasia of the adrenals in males (54% vs. 35% in the control).

At 1000 ppm, there was an increased incidence of tactile hair loss in males (23% vs. 0% in the control), and there was an increasing trend of this sign in females (40% vs. 21% in the control). A significant increase in incidences of fur loss in females was suspected of being treatment-related (58% vs. 21% in the control).

Mean body weights of males in the 1000 ppm group were significantly lower than the control at Week 2 (↓4%), and from Week 4 to the end of the treatment period (↓5-17%). Females in the 1000 ppm group

had significantly lower mean body weights at Weeks 4 and 5 (↓5%), and from Week 7 to the end of the treatment period (↓7-26%).

At termination of treatment at 1000 ppm, the mean body weights of males and females in the 1000 ppm group were 89% and 74% of the control values, respectively. A non-statistically significant increase in mean relative liver weight was found in males (↑30%), and in females there was a significant increase in both mean absolute and relative liver weights (↑34% and ↑67%). Finally there was a significant increase in relative weight of kidneys in females (↑40%).

At 1000 ppm, there was a significant increase in incidences of seminiferous tubule atrophy in males that were killed *in extremis* or found dead. There was also a significant decrease in mean absolute epididymide weights observed in males which was thought to be related to the testicular lesions (↓28%). There was a significant increase in incidences of mammary gland hyperplasia in females (37% v. 9% in the control), and a significant increase in incidences of endometrial hyperplasia of the uterine horn of females (63% vs. 44% in controls). Statistically significant changes associated with the liver were increases in incidences of centrilobular hypertrophy and cell necrosis of hepatocytes in both sexes (81% for males and 88% in females vs. 0% in the controls), and increases of incidences of focal necrosis of hepatocytes (50-61% for both sexes) and dark-colored liver (17% vs. 0% in the control), coarse surface (12% vs. 0% in the control), and spots seen at necropsy in males (15% vs. 0% in the control).

At 1000 ppm, there were significant increases in incidences of follicular cell hypertrophy of the thyroid in both sexes (21% in males and 42% in females vs. 0% in the controls). A significant increase in incidences of subcapsular cell hyperplasia of the adrenals was observed in males (62% vs. 35% in the control). Increased incidences of diffuse acinar cell atrophy in the pancreas were noted in females (15% vs. 0% in the control). Significant increases in incidences of intracytoplasmic eosinophilic bodies in respiratory epithelial cells (29-40% for both sexes vs. 4-21% in the controls) and in olfactory cells (males, 54% vs. 9% in controls) were observed.

Finally at the 1000 ppm concentration, there were significant increased incidences of benign interstitial (leydig) cell tumors (23% vs. 0 in the control), interstitial (leydig) cell hyperplasia (17% vs. 0 in the control), and masses (12 % vs.0 in the control) in the testes in males. Tumor increases were considered to be a secondary effect resulting from anti-androgenic activity of the test substance and not a result of a direct carcinogenic property of the test substance.

The systemic LOAEL for mice is 250 ppm (equivalent to 27.1 mg/kg/day for males, 25.0 mg/kg/day for females), based on decreased mean body weight in males; and increased incidences of tactile hair loss in males, endometrial hyperplasia of the uterine horn in females, follicular cell hypertrophy of the thyroid in males, and subcapsular cell hyperplasia of the adrenal in males. The NOAEL is 60 ppm (equivalent to 6.25 mg/kg/day for males and 5.82 mg/kg/day for females).

At the doses tested, there was a treatment-related increase in the incidence of benign interstitial (leydig) cell tumors in males in the 1000 ppm treatment group when compared with the control.

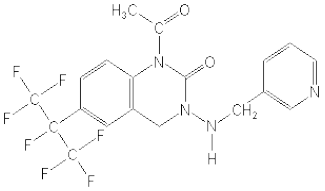
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200) for a carcinogenicity study in mice.

COMPLIANCE: A signed and dated No Data Confidentiality statement was provided. Dated GLP and Quality Assurance statements were provided; the Reviewer's copies of these statements were not signed, but were said to have been signed in the originals. The data were claimed to be the property of Nichino America Inc. and considered to be confidential for all purposes other than compliance with FIFRA §10. The study was conducted in accordance with USEPA, FIFRA GLP Standards, 40 CFR Part 160 (1989); MAFF in Japan, The Standards of GLP for Agricultural Chemicals, 11-Nousan-No. 6283 (1999); and OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17 (1998).

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: NNI-0101 Technical

Description:	Not provided
Lot/batch #:	3FZ0013G
Purity:	98.0%
Compound stability:	Expiration date: September 20, 2007
CAS # of TGAI:	337458-27-2
Structure:	

2. Vehicle control: The vehicle for the test substance was the basal diet (see details below).

3. Test animals:

Species:	Mouse
Strain:	Specific-pathogen-free (SPF) ICR (Crj:CD-1)
Age/weight at study initiation:	5 weeks (both sexes); 28.1 to 34.6 grams for males, 22.8 to 30.2 grams for females
Source:	Atsugi Breeding Center, Charles River Japan, Inc. (Kanagawa Japan)
Housing:	Aluminum cages with wire-mesh floors (215 mm wide, 330 mm deep, 180 mm high) on movable stainless steel racks; 4 animals of same sex and dose group per cage after grouping
Diet:	Certified Diet, MF Mash (Oriental Yeast Co., Ltd; Tokyo, Japan), *ad libitum* in stainless steel feeding jars; each dietary lot analyzed for nutrient content by supplier and for contaminants by Japan Food Research Laboratories (Tokyo) prior to shipment; food changed within <4 days
Water:	Well water passed through a rapid filtration unit with sand filter and an adsorption unit with charcoal filter, sterilized with sodium hypochlorite and ultraviolet light; *ad libitum* from plastic bottles
Environmental conditions:	**Temperature:** Controlled at 23 ± 2EC; 7 deviations, ranging from –0.1 to +0.4EC (within 1 hour or less) were recorded **Humidity:** Controlled at 55 ± 15% **Air changes:** 10/hr (minimum), fresh air **Photoperiod:** 12 hrs dark/ 12 hrs light (artificial illumination) Because deviations of temperature and humidity were temporary, and no abnormalities were observed in the animals after the incidents, they were not considered to have affected the outcome of the study.
Acclimation period:	9 days, males; 10 days, females

B. STUDY DESIGN:

1. In life dates:
Start: June 3, 2004 (initiation of treatment)
End: end of treatment not stated; assumed by Reviewer to be in December 2005

2. Animal assignment/dose levels: Animals were randomly assigned to the test groups shown in Table 1. Animals were exposed to the test substance in the diet for 18 months (78 weeks).

Animals were assigned to each dose group on the day of treatment initiation through a stratified

random sampling method based on body weights. After allocation, it was confirmed that there were no statistically significant differences in mean body weights among groups and that individual body weights were within 80-120% of the mean value of each sex.

TABLE 1. Study design

Group	Concentration of NNI-0101 in the diet (ppm)	Number of Animals	
		Males	Females
Control	0	52	52
Low-dose	60	52	52
Mid-dose	250	52	52
High-dose	1000	52	52

3. **Dose selection rationale:** The dose levels used in the present study were selected on the basis of a previous study (IET 03-0063[1]), in which the test substance was administered to SPF ICR (Crj:CD-1) mice (10/sex) in the diet for 90 days at a dietary concentration of 0, 60, 750, or 1500 ppm. Among the findings in the 1500 dose group were the following: significant decrease in food consumption (both sexes); anemia (both sexes), significant increase (males) in reticulocyte count and significant decreases in total leukocyte count, and lymphocytes and eosinophils in differential leukocyte count; significant increases (both sexes) in liver-related blood chemistry parameters and total bilirubin and significant decrease (both sexes) in blood glucose; significant fluctuations in blood lipid and protein (both sexes) and blood electrolytes (males); significant increases in weights of the liver, thyroid, spleen (both sexes) and adrenals (males); significant decrease in epididymis weight (males); significant increases (both sexes) in incidences of centrilobular hepatocellular hypertrophy, focal hepatocellular necrosis, cell infiltration in the liver, and follicular cell hypertrophy of the thyroid; significant increases (males) in incidences of congestion and increased extramedullary hematopoiesis in the spleen, interstitial cell hyperplasia in the testis, and diffuse cortical cell vacuolation and subcapsular cell hyperplasia in the adrenal; significant increase in incidences of atrophy of the ovary (females).

   Among the findings in the 750 ppm dose group were the following: significant decreases (males) in total leukocyte count and lymphocytes; anemia (females); significant increases (males) in liver-related parameters; significant decreases (males) in protein and electrolytes; significant increase in liver weight (both sexes) and thyroid weight (males); significant decrease in epididymis weight (males): significant increases (both sexes) in incidences of centrilobular hepatocellular hypertrophy and follicular cell hypertrophy in the thyroid.

   In the 60 ppm group, there were no treatment-related changes in either sex.

4. **Diet preparation and analysis:** The dosing formulations were prepared once prior to the initiation of treatment and approximately once every 2 weeks during the treatment period. To prepare the formulations, a required amount of the test substance was mixed with basal diet in a mortar to provide a premix for each dose level. The premix was then added to the rest of the basal diet and they were blended using a blending machine. The amount of test substance mixed with the basal diet was not corrected for the purity of the test substance. The prepared diets were sealed in plastic bags and

[1] NNI-0101 Technical: Repeated dose 90-day oral toxicity study in mice (Dose range-finding study for a 18-month carcinogenicity study (IET 03-0063), Final Report, The Institute of Environmental Toxicology, 2005.

stored in aluminum containers at approx. 4º C until use.

Chemical analyses for homogeneity and test substance concentration were performed on samples (approx 50 g) from the top, middle, and bottom portions in the mixer from each dose level (including the control diet) collected at the first preparation, at approximately 6 and 12 months after the initiation of the experiment, and at the last preparation. Test substance concentrations in all test diets also were determined on samples (50 g) collected after use at approximately 3, 9, and 15 months after treatment initiation to ensure proper formulation and storage procedures were being followed.

Stability of the test substance in the diet at concentration levels of 10 and 4000 ppm were verified in a prior study.[2] Samples of the 4000 ppm diet kept under sealed, cold, and dark conditions for 35 days, and then exposed to ambient air in an animal room for 14 or 21 days were stated to have been close to an initial value. Decreasing trends in concentrations of the test substance in the 10 ppm diets were observed over time; however, 85% of the test substance remained when kept under sealed, cool, dark conditions for 35 days and then exposed to ambient air in an animal room for 7 days. Therefore, diets prepared for study use were defined to be stored under sealed, cold, dark conditions for 21 days and then exposed to ambient air in an animal room for 5 days to ensure the stability of the diet.

Analytical methods were in accordance to the report on validation of analytical procedures for NNI-0101 (active ingredient) in the diet.[3] The test substance was extracted from the diet with acetonitrile and analyzed using high performance liquid chromatography (HPLC). Samples were analyzed in duplicate.

Results:

Homogeneity analysis: 0.36-5.2% (RSD)

Stability analysis: 90-105 % of initial values for 60 ppm after 14 days, 91-97 % of initial values for 60 ppm after 14 days , and 93-99 % of initial values for 1000 ppm after 14 days.

Concentration analysis: The actual average concentrations of test material in individual diets were 58.3, 245.13 and 989.3 ppm for the 60, 250, and 1000 ppm dose levels, respectively, and ranged from 96 to 100% of the targeted values. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** The data on body weight, food consumption, total and differential leukocyte counts, and organ weights were evaluated by Barlett's test for equality of variance. When group variances were homogeneous, a parametric analysis of variance (ANOVA) of a one-way type layout was performed to determine if there were statistical differences among group means. When a significant difference among group means was indicated by ANOVA, Dunnett's multiple comparison test was conducted. When Bartlett's test indicated that group variances were heterogeneous, the data were evaluate by Kruskal-Wallis non-parametric analysis of variance. If significant, the Dunnett-type mean rank sum

[2] NNI-0101 Technical: Stability study in the diet for rodent (IET 02-5019), Final Report, The Institute of Environmental Toxicology, 2004.
[3] Validation of analytical procedures for NNI-0101 Technical (active ingredient) in diet (IET 03-5032), Final Report, The Institute of Environmental Toxicology, 2003.

test was applied.

Life table analysis was used for comparison of mortality. General clinical observations and gross pathological and histopathological lesions were evaluated using Fisher's exact probability test (one-tail analysis).

C. METHODS:

1. **Observations:** Cage-side observations for mortality and morbidity were performed on all animals at least twice a day (at lead once daily on weekends and national holidays). A general clinical observation was performed on all animals once daily, in the morning. A clinical examination, including palpation, was conducted at least once a week. Animals were observed in the home cage for excitement, sedation, abnormal posture, and abnormal behavior. Animals were observed during handling for difficulty in handling, changes in muscle tone, tremors, palpebral closure, salivation, lacrimation, discharges from orifices, exophthalmos, changes in body temperature, abnormal respiratory sounds, changes in fur, and changes in skin and visible mucous membranes. Outside the cage, animals were observed for jumping, circling, convulsions, abnormal gait, change in spontaneous motor activity, change in respiration, vocalization, piloerection, abnormal posture, and abnormal behavior.

2. **Body weight:** Body weights were recorded at the initiation of treatment (Week 0, before feeding of the test substance), once a week from Week 1 to 13, once every 4 weeks from Week 16 to 76, and at the termination of treatment (Week 78). Final body weights were recorded for all animals before euthanasia or when found dead.

3. **Food consumption and compound intake:** Food consumption was recorded, by cage, once weekly from Week 1 to 13, and every 4 weeks from Week 16 for a period of 4 consecutive days. Mean daily food consumption per animal per cage per day was calculated and used to calculate weekly group mean food consumption (gram/animal/day). The weighted average of the group mean food consumption throughout the treatment period also was calculated for each dose group of each sex.

   For each week of measurement, group mean test substance intake (mg/kg/day) was calculated for each dose group of each sex by multiplying the group mean food consumption by the nominal dietary concentration, and dividing by the group mean body weight. The weighted average of the group means test substance intakes throughout the treatment period also were calculated.

4. **Hematology and clinical chemistry:** All surviving animals after 78 weeks of treatment were laparotomized under ether anesthesia, and blood samples were collected from the posterior vena cava using heparinized syringes. An aliquot of each sample was transferred to a cup treated with EDTA and the following parameters were evaluated:

	Hematocrit (HCT)	X	Leukocyte differential count*[a]
	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)		Mean corpusc. HGB conc. (MCHC)
	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for carcinogenicity studies (Cont. and HDT unless effects are observed) based on Guideline 870.4200 & OECD 451

[a] lymphocyte, neutrophil, monocyte, eosinophil, basophil, large unstained cell

Blood smears stained with May-Grunwald and Giemsa also were prepared from all samples. When the large unstained cell count was 0.3 x 10^3/µL or greater, or there were any parameters not successfully determined by the hematology analyzer for an animal, the blood smears for the animal was examined microscopically for the presence of leukemic cells.

Blood smears also were prepared from all surviving animals after 52 weeks of treatment and stained with May-Grunwald and Giemsa. Blood samples were obtained by cutting the tip of the tail of each animal while under anesthesia. In addition, blood smears were prepared from animals killed *in extremis* during the treatment period using the same method as that used for surviving animals at Week 52. These smears were stained with Sangodiff® or May-Grunwald and Giemsa, and the percentage of leukocyte types was determined microscopically.

Other clinical chemistry parameters (e.g., blood electrolytes and enzymes) were not evaluated in the present study.

5. **Sacrifice and pathology:** Necropsy was performed on all animals, including those found dead during the treatment period. Animals killed at the scheduled time after 78 weeks of treatment were euthanized under deep ether anesthesia by exsanguination from the abdominal aorta and posterior vena cava. Animals found dead during the treatment period were necropsied immediately after discovery. Tissues and organs in the table below designated with an "X" were collected at necropsy from all animals and fixed in 10% neutral-buffered formaldehyde solution. The lungs were prefixed by instillation through the trachea before fixation. Of the preserved tissues and organs, those examined histologically from all animals in the 0 and 1000 ppm groups, and from animals in the 60 and 250 ppm groups found dead during treatment, are designated by the superscript "a". Those organs and tissues examined histologically from animals killed at termination in the 60 and 250 ppm groups are designated by the superscript "b". Organ weights were measured at necropsy after 78 weeks of treatment (10 animals/sex/dose group, selected in order of animal numbers). The organs that were weighed are designated by "Y" in the following table. The liver weight included the gall bladder; the thyroid weight included the parathyroids.

	DIGESTIVE SYSTEM		CARDIOVASC. / HEMAT.		NEUROLOGIC
X	Tongue	X[a]	Aorta, thoracic*	X[a]Y	Brain (multiple sections)*+ (cerebrum, cerebellum, pons/medulla)
X[a]	Salivary glands* (submaxillary and sublingual)	X[a]Y	Heart*+	X[a]	Peripheral nerve* (sciatic, unilateral)
X[a]	Esophagus*	X[a]	Bone marrow* (with bone: sternum; femur, unilateral; cervical, thoracic and lumbar vertebrae)	X[a]	Spinal cord (3 levels)* (cervical, thoracic, lumbar)
X[a]	Stomach* (forestomach and glandular stomach)	X[a]	Lymph nodes*(cervical and mesenteric)	X[a]	Pituitary*
X[a]	Duodenum*	X[a]Y	Spleen*+	X[a]	Eyes (retina, optic nerve)*
X[a]	Jejunum*	X[a]	Thymus		
X[a]	Ileum*				**GLANDULAR**
X[a]	Cecum*			X[abc]Y	Adrenal gland*+
X[a]	Colon*		**UROGENITAL**		Lacrimal gland
X[a]	Rectum*	X[a]Y	Kidneys*+	X[a]	Parathyroids*
X[ab]Y	Liver*+	X[a]	Urinary bladder*	X[ab]Y	Thyroids*
X[a]	Gall bladder* (not rat)	X[ab]Y	Testes*+	X[a]	Harderian glands
	Bile duct* (rat)	X[ab]Y	Epididymides*+		
X[abd]	Pancreas*	X[a]	Prostate*		**OTHER**
	RESPIRATORY	X[ab]	Seminal vesicle*	X[a]	Bone (with bone marrow: sternum; femur, unilateral; cervical, thoracic and lumbar vertebrae)
X[a]	Trachea*	X[ab]	Coagulating glands	X[a]	Skeletal muscle (M. triceps surae, unilateral)
X[a]	Lungs* (including bronchi)	X[a]Y	Ovaries*+	X[a]	Skin* (lumbodorsal region)
X[ab]	Nose* (nasal cavity)	X[abe]Y	Uterus*+ (including cervix)	X[ab]	All gross lesions and masses*
X[a]	Pharynx*	X[abf]	Mammary gland* (abdominal region)	X[a]	Knee joint (unilateral)
X[a]	Larynx*	X[a]	Vagina	X	Head (including nasal cavity and paranasal sinuses, oral mucosa, and middle ears)

X = collected from all animals at necropsy and fixed in preservative
Y = organ/tissue weighed
* Required for carcinogenicity studies based on Guideline 870.4200.
+ Organ weight required in carcinogenicity studies.

a Tissues and organs histologically examined from all animals in the 0 and 1000 ppm groups, and from animals in the 60 and 250 ppm groups found dead during treatment.

b Organs and tissues histologically examined from animals killed at termination in the 60 and 250 ppm groups.

c In the 60 and 250 ppm groups killed at study termination, only males were examined.

d In the 60 and 250 ppm groups killed at study termination, only females were examined.

e Uterine horn only in females in the 60 and 250 ppm groups killed at study termination.

f Females only.

II. RESULTS:

A. OBSERVATIONS:

1. Clinical signs of toxicity: Males in the 250 and 1000 ppm dose groups had significant increases in fur loss; however, the incidence of fur loss was not dose dependent. In the 1000 ppm dose group, females also had a statistically significant increase in incidences of fur loss (62% vs. 38% in the control). Compared to the control group, females showed an increasing trend in incidences of fur loss in the head and perinasal regions; there were no specific sites where fur loss was frequently observed in males

A significant increase in incidences of tactile hair loss was seen in males in the 250 and 1000 ppm dose groups (23% and 29%). An increase in incidences of tactile hair loss also was seen in females in the 1000 ppm group when compared with the control, but statistical significance was not shown (44% vs. 27% in the control).

Females in the 250 ppm dose group showed a significant increase in incidences of pale-colored skin (13%) but this effect was not dose dependent. Males in the 1000 ppm dose group had significant increases in incidences of wetted fur and abdominal distention (35% vs. 18% in the control), and a significant decrease in slough formation in the skin. Males in the 60 ppm dose group had significant decreases in incidences of bradypnea (9% vs. 25% in the control) and dark eye color (0% vs. 9% in the control).

The statistically significant clinical findings are summarized in Table 2.

TABLE 2. Statistically significant clinical findings (all animals)[a]

	Number of findings							
	Males				Females			
Concentration (ppm) of NNI-0101 in diet: (n)	**0 (51)**	**60 (52)**	**250 (52)**	**1000 (52)**	**0 (52)**	**60 (52)**	**250 (52)**	**1000 (52)**
Clinical Finding								

Posture/body position Abdominal distention	9 (18%)	7	11	18* (35%)	1	1	0	1
Respiration Bradypnea	13 (25%)	5* (9%)	12	12	8	5	11	5
Skin Slough formation Pale coloration	7 7	8 2	5 7	0** 2	0 1	0 3	0 7* (13%)	0 4
Fur Tactile hair loss Loss of fur Wetted fur	0 3 1	2 9 4	12** (23%) 18** (35%) 2	15** (29%) 12* (23%) 12** (23%)	14 (27%) 20 (38%) 1	9 19 0	13 14 0	23 (44%) 32* (62%) 0
Eye Dark color	5	0*	3	2	1	2	3	2

[a] Data were obtained from page 24 (Text Table 1) of the study report, 56-59 (Raw Data)
* Significantly different from the control ($p \leq 0.05$) using Fisher's exact test.
** Significantly different from the control ($p \leq 0.01$) using Fisher's exact test.

2. **Mortality:** There were no significant changes in mortality in either sex during the study. The mortality at the end of 78 weeks of treatment was 17/51, 19/52, 20/52, and 20/52 for males; and 10/52, 14/52, 14/52, and 9/52 for females in the 0, 60, 250, and 1000 ppm dose groups, respectively. One male in the control group was found dead between a cage edge and cover during Week 3; this death was considered to be "accidental" and was not included in the final mortality statistics.

B. BODY WEIGHT:

Mean body weights of both sexes in the 60 ppm dose group were comparable to those of controls throughout the treatment period, with the exception of a significant reduction in males at Weeks 8 and 24. At termination of treatment, the mean body weights of males and females in the 60 ppm treatment group were 99% and 105% of the control values, respectively.

Mean body weights in males of the 250 ppm dose group were significantly lower than the control values from Weeks 8 to 56 (↓5-8%). The mean body weight of females in the 250 ppm dose group were comparable to the controls throughout the treatment period, with the exception of a significant decrease at Week 4. At termination of treatment, the mean body weights of males and females in the 250 ppm treatment group were 99% and 100% of the control values, respectively.

Mean body weights of males in the 1000 ppm group were significantly lower than the control at Week 2 (↓4%), and from Week 4 to the end of the treatment period (↓5-17%). Females in the 1000 ppm group had significantly lower mean body weights at Weeks 4 and 5 (↓5%), and from Week 7 to the end of the treatment period (↓7-26%). At termination of treatment, the mean body weights of males and females in the 1000 ppm group were 89% and 74% of the control values, respectively.

Body weight gains were not calculated.

Mean body weights during the treatment period are summarized in Table 3.

TABLE 3. Average body weight during the treatment period[a]

Concentration of NNI-0101 in diet (ppm):	Mean body weight (grams±SD) (n)							
	Males (n)				Females			
	0	60	250	1000	0	60	250	1000
Week								
0	31.4 ±1.6 (52)	31.4 ±1.6 (52)	31.4 ±1.6 (52)	31.4 ±1.6 (52)	25.8 ±1.7 (52)	25.8 ±1.7 (52)	25.8 ±1.7 (52)	25.8 ±1.7 (52)
1	34.6 ±1.9 (52)	34.6 ±2.2 (52)	34.6 ±1.9 (52)	34.5 ±1.9 (52)	27.3 ±1.9 (52)	27.0 ±1.9 (52)	27.1 ±1.9 (52)	27.3 ±1.8 (52)
2	37.4 ±2.2 (52)	36.5 ±2.4 (52)	36.9 ±2.3 (52)	36.1* ±2.0 (52) (↓4%)	29.1 ±2.1 (52)	28.6 ±2.3 (52)	28.8 ±2.2 (52)	28.8 ±1.9 (52)
3	38.5 ±2.4 (51)	37.7 ±2.9 (52)	38.0 ±2.6 (52)	37.4 ±2.2 (52) (↓3%)	30.5 ±2.4 (52)	29.9 ±2.6 (52)	29.9 ±2.2 (52)	29.6 ±2.2 (52)
4	40.2 ±2.7 (51)	39.0 ±2.8 (52)	39.4 ±2.9 (52)	38.5** ±2.2 (52) (↓5%)	32.4 ±3.3 (52)	31.3 ±2.7 (52)	30.9* ±2.7 (52) (↓5%)	30.8* ±2.6 (52) (↓5%)
5	40.8 ±2.8 (51)	39.8 ±2.9 (52)	40.1 ±2.9 (52)	39.3* ±2.3 (52) (↓4%)	32.8 ±2.8 (52)	31.6 ±3.0 (52)	31.7 ±2.9 (52)	31.3* ±2.4 (52) (↓5%)
6	42.0 ±3.2 (51)	41.0 ±3.4 (52)	41.0 ±3.3 (52)	39.8** ±2.3 (52)	33.6 ±3.3 (52)	32.7 ±3.5 (52)	33.3 ±3.3 (52)	32.0 ±2.6 (52) (↓5%)
7	43.5 ±3.5 (51)	42.3 ±3.6 (52)	42.1 ±3.7 (52)	40.4** ±2.2 (52) (↓6%)	35.0 ±3.8 (52)	34.0 ±3.7 (52)	33.8 ±3.3 (52)	32.7** ±2.5 (52) (↓7%)
8	44.6 ±3.6 (51)	42.8* ±4.0 (52)	42.6** ±3.7 (52) (↓5%)	41.1** ±2.2 (52) (↓8%)	36.2 ±3.9 (52)	35.2 ±4.3 (52)	34.8 ±3.7 (52)	33.1** ±2.5 (52) (↓9%)
9	45.1 ±3.9 (51)	43.5 ±4.0 (52)	43.1* ±3.8 (52) (↓5%)	41.4** ±2.4 (52) (↓9%)	35.9 ±4.2 (52)	35.1 ±4.3 (52)	35.5 ±4.4 (52)	33.0** ±2.7 (52) (↓11%)
10	46.1 ±4.1 (51)	44.5 ±4.4 (52)	44.0* ±4.1 (52) (↓5%)	42.0** ±2.6 (52) (↓9%)	37.3 ±4.6 (52)	36.3 ±4.7 (52)	36.4 ±4.1 (52)	33.3** ±2.8 (52) (↓11%)
11	47.1 ±4.5 (51)	45.6 ±4.6 (52)	44.4** ±4.0 (52) (↓6%)	42.1** ±2.5 (52) (↓9%)	37.7 ±4.5 (52)	36.8 ±5.0 (52)	37.7 ±4.7 (52)	33.7** ±2.9 (52) (↓11%)
12	47.9 ±4.4 (51)	46.3 ±4.9 (52)	44.9** ±4.0 (51) (↓7%)	42.6** ±2.5 (52) (↓12%)	38.7 ±4.8 (52)	38.2 ±5.1 (52)	38.8 ±5.0 (52)	34.2** ±3.2 (52) (↓12%)
13	48.0 ±4.7 (50)	46.6 ±4.9 (52)	45.3* ±4.1 (51) (↓6%)	42.6** ±2.4 (52) (↓12%)	39.5 ±5.2 (52)	38.2 ±5.3 (52)	39.0 ±4.8 (52)	34.2** ±3.3 (52) (↓14%)
16	50.1 ±4.9 (50)	48.3 ±5.4 (52)	46.7** ±4.8 (51) (↓7%)	43.6** ±2.7 (52) (↓13%)	42.4 ±5.8 (52)	41.0 ±5.7 (52)	41.7 ±5.7 (52)	35.1** ±3.7 (52) (↓18%)
20	51.2 ±5.1 (50)	49.1 ±6.0 (52)	47.9** ±5.3 (51) (↓7%)	44.2** ±3.2 (52) (↓14%)	44.3 ±6.3 (52)	43.6 ±6.6 (52)	44.1 ±6.1 (52)	36.4** ±4.1 (52) (↓18%)
24	52.4 ±5.3 (50)	49.8* ±6.1 (52)	48.7** ±5.3 (51) (↓7%)	44.3** ±2.8 (51) (↓16%)	46.4 ±6.0 (52)	45.1 ±6.9 (52)	46.5 ±6.6 (52)	37.2** ±4.5 (52) (↓20%)
28	53.2 ±5.8 (50)	50.8 ±6.3 (52)	49.4** ±5.2 (51) (↓8%)	44.8** ±3.1 (51) (↓16%)	47.6 ±6.7 (52)	47.2 ±7.1 (51)	47.5 ±7.3 (51)	37.8** ±4.4 (51) (↓21%)
32	53.3 ±5.7 (50)	51.1 ±6.3 (51)	49.2** ±5.5 (51)	44.9** ±3.1 (51)	48.5 ±7.2 (51)	48.3 ±8.0 (50)	48.9 ±7.6 (50)	38.5** ±4.8 (51)

TABLE 3. Average body weight during the treatment period[a]								
Concentration of NNI-0101 in diet (ppm):	Mean body weight (grams±SD) (n)							
	Males (n)				Females			
	0	60	250	1000	0	60	250	1000
Week								
			(↓8%)	(↓16%)				(↓21%)
36	54.4 ±5.9 (49)	52.2 ±6.1 (51)	50.1** ±5.4 (49) (↓8%)	45.2** ±3.3 (50) (↓17%)	50.8 ±6.6 (50)	49.9 ±7.8 (50)	49.8 ±7.9 (50)	39.3`** ±4.9 (51) (↓21%)
40	54.8 ±5.9 (48)	52.9 ±6.5 (50)	50.6** ±5.8 (49) (↓8%)	45.5** ±3.0 (50) (↓17%)	52.0 ±6.9 (50)	51.1 ±8.3 (50)	50.9 ±7.6 (50)	39.6** ±5.0 (51) (↓21%)
44	54.6 ±5.7 (47)	52.3 ±6.5 (49)	50.8** ±5.7 (49) (↓7%)	45.6** ±3.3 (50) (↓17%)	51.9 ±7.0 (50)	52.1 ±7.8 (50)	51.3 ±7.9 (49)	39.5** ±5.3 (50) (↓24%)
48	54.0 ±5.6 (47)	52.7 ±6.6 (48)	50.8* ±5.7 (49) (↓6%)	45.9** ±3.6 (50) (↓17%)	52.7 ±6.9 (49)	53.1 ±8.3 (50)	52.1 ±8.1 (49)	39.8** ±5.0 (50) (↓26%)
52	54.0 ±6.3 (46)	52.3 ±6.8 (47)	50.4* ±5.9 (48) (↓7%)	45.2** ±3.9 (49) (↓17%)	53.8 ±7.0 (49)	53.1 ±8.5 (50)	52.4 ±8.1 (49)	40.3** ±4.9 (50) (↓25%)
56	54.2 ±6.3 (43)	52.3 ±6.2 (45)	50.4* ±5.7 (48) (↓8%)	45.4** ±3.3 (47) (↓17%)	53.5 ±6.7 (49)	53.5 ±8.7 (49)	52.2 ±8.9 (48)	40.1** ±5.3 (49) (↓25%)
60	53.5 ±6.9 (43)	51.9 ±6.2 (43)	50.4 ±4.9 (46) (↓8%)	45.3** ±3.5 (45) (↓16%)	53.9 ±7.0 (47)	53.6 ±9.1 (48)	51.8 ±9.2 (45)	40.5** ±5.0 (47)
64	53.2 ±6.2 (41)	51.9 ±6.2 (41)	50.1 ±5.1 (46)	45.9** ±3.2 (42) (↓14%)	54.0 ±7.1 (47)	53.0 ±8.8 (47)	52.5 ±8.6 (41)	40.7** ±4.8 (46) (↓25%)
68	52.8 ±6.4 (40)	52.1 ±6.6 (38)	49.5 ±4.9 (44)	44.6** ±3.6 (41) (↓14%)	54.0 ±7.3 (47)	53.0 ±9.3 (45)	52.9 ±8.8 (41)	40.6** ±4.6 (45) (↓25%)
72	51.2 ±6.4 (38)	52.9 ±6.6 (34)	49.3 ±5.3 (40)	44.4** ±3.6 (37) (↓14%)	54.0 ±7.1 (46)	53.2 ±9.1 (40)	53.9 ±8.8 (39)	40.4** ±4.6 (44) (↓26%)
76	51.1 ±7.1 (37)	53.7 ±6.8 (33)	49.3 ±4.8 (36)	44.7** ±3.7 (34) (↓13%)	53.5 ±7.1 (44)	53.0 ±9.3 (38)	53.4 ±8.7 (39)	40.1** ±4.5 (43) (↓26%)
78	50.9 ±7.4 (34)	53.2 ±7.1 (33)	50.2 ±4.6 (32)	45.2** ±3.6 (32) (↓12%)	53.3 ±6.9 (42)	52.6 ±9.8 (38)	53.5 ±9.1 (38)	39.6** ±4.3 (43) (↓26%)

[a] Data were obtained from pages 60 and 61 [Table 5 (1-2)], pages 62 and 63 [Table 6 (1-2)], and pages 196-227 (Raw Data) of the study report.
*Significantly different from the control ($p \leq 0.05$).
** Significantly different from the control ($p \leq 0.01$).

C. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE:

1. **Food consumption:** When compared with their controls, overall food consumption for males and females, respectively, was 100% and 102% for the 60 ppm dose group, 100% and 105% for the 250 ppm dose group, and 103% and 102% for the 1000 ppm dose group. In the 60 ppm dose group, there was a significant increase in food consumption by females at Week 40. In the 250 ppm dose group, there was a significant decrease in food consumption by females at Week 1, and significant increases at Weeks 11 and 40. In the 1000 ppm dose group, there were significant increases in food consumption by males at Weeks 9, 13, and 20 and by females at Weeks 5 and 40; and significant decreases by females at Weeks 1 and 3. Text tables are presented on pages 64-67, and raw data are

located in pages 228-235 of the study report.

2. Test substance consumption: The average test substance intakes are summarized in Table 4.

TABLE 4. Average test substance consumption during the treatment period[a]

Concentration of NNI-0101 in diet (ppm)	Average test substance consumption (mg/kg/day)	
	Males	Females
60	6.25	5.82
250	27.1	25.0
1000	122	120

[a] Data were obtained from page 26 (Text Table 2), and pages 68-69 (Raw Data) of the study report.

D. BLOOD ANALYSES (HEMATOLOGY):

There were no statistically significant changes in any evaluated blood parameters in any treatment group of either sex after 78 weeks of treatment. Because no treatment-related changes such as an increase in neoplasms in the hematopoietic system were detected after 78 weeks of treatment (see results below), the blood smears prepared after 2 weeks of treatment were not analyzed.

E. SACRIFICE AND PATHOLOGY:

1. Organ weights: There was a significant decrease in the mean absolute brain weight in males in the 250 and 1000 ppm treatment groups (↓6-7%). In females, there was a significant increase in mean absolute brain weight in the 60 ppm (↑5%) dose group and a significant increase in relative brain weight in the 1000 ppm group (↑25%).

A significant increase in mean relative liver weight was found in both sexes in the 1000 ppm dose group (↑51% for males and ↑67% for females). In females, there was a significant increase in mean absolute liver weights in the 1000 ppm group (↑34%); an increasing tendency in males was noted.

A significant increase in mean relative heart weight was seen in both males and females in the 1000 ppm group (↑16% for males and ↑26% for females). Also in the 1000 ppm dose group, a significant decrease in mean absolute epididymides (↓28%) weight in males and a significant increase in the mean relative kidney weight of females were observed (↑40%).

Tables 5 and 6 summarize, respectively, the mean absolute and relative weights of those organs showing a statistically significant change in mean absolute and/or relative weight in one or both sexes at one or more dose levels. Table 7 compares all significant changes to the control value (%).

TABLE 5. Average terminal body weights and selected absolute organ weights[a]

Concentration of NNI-0101 in diet (ppm)	Mean terminal body weight (g±SD)	Mean absolute organ weight					
		Brain (mg±SD)	Liver (g±SD)	Heart (mg±SD)	Thyroids (mg±SD)	Kidneys (mg±SD)	Epididy-mides (mg±SD)
Males (n=10)							

0 (control)	52.4 ±5.7	518 ±26	2.82 ±0.71	228 ±22	5.5 ±1.5	836 ±123	121 ±17
60	54.4 ±8.4	513 ±18	2.75 ±0.51	238 ±32	5.4 ±1.7	897 ±127	121 ±8
250	49.2 ±4.3	488** ±15 (↓6%)	2.46 ±0.44	229 ±40	4.8 ±1.6	797 ±181	103 ±20
1000	45.6* ±4.4	482** ±24 (↓7%)	3.66 ±1.38	232 ±31	6.0 ±1.8	817 ±282	88** ±23 (↓28%)
Females (n=10)							
0 (control)	50.3 ±4.5	509 ±13	1.97 ±0.25	171 ±21	4.6 ±1.2	489 ±61	-
60	55.6 ±13.3	537** ±24 (↑5%)	2.18 ±0.57	183 ±25	3.9 ±1.1	524 ±82	-
250	52.2 ±6.7	515 ±23	2.44 ±0.42	189 ±14	4.9 ±1.1	557 ±43	-
1000	40.1** ±4.4	502 ±11	2.63** ±0.49 (↑34%)	173 ±24	4.7 ±1.3	540 ±73	-

[a] Data were obtained from page 90 (Table 15-1), page 92 (Table 16-1), and pages 257-258 and 261-262 (Raw Data) of the study report.
*Significantly different from the control ($p \leq 0.05$).
** Significantly different from the control ($p \leq 0.01$).

TABLE 6. Average selected relative organ weights[a]

Concentration of NNI-0101 in diet (ppm)	Mean relative organ weight[b]					
	Brain (%)	**Liver (%)**	**Heart (%)**	**Thyroids (%)**	**Kidneys (%)**	**Epididy-mides (%)**
Males (n=10)						
0 (control)	1.00 ±0.10	5.37 ±1.20	0.44 ±0.04	0.0105 ±0.0024	1.60 ±0.21	0.23 ±0.04
60	0.96 ±16	5.07 ±0.78	0.44 ±0.05	0.0102 ±0.0037	1.67 ±0.29	0.23 ±0.04
250	1.00 ±0.09	5.02 ±1.02	0.47 ±0.09	0.0098 ±0.0035	1.63 ±0.36	0.21 ±0.04
1000	1.07 ±0.12	8.09* ±3.21 (↑51%)	0.51* ±0.06 (↑16%)	0.0131 ±0.0037	1.77 ±0.43	0.19 ±0.04
Females (n=10)						
0 (control)	1.02 ±0.10	3.93 ±0.36	0.34 ±0.03	0.0092 ±0.0027	0.97 ±0.09	-
60	1.02 ±0.28	3.98 ±0.74	0.34 ±0.07	0.0072 ±0.0016	0.99 ±0.31	-
250	1.00 ±0.15	4.72 ±0.88	0.37 ±0.04	0.0094 ±0.0020	1.08 ±0.10	-
1000	1.27** ±0.13 (↑25%)	6.59** ±1.18 (↑67%)	0.43** ±0.06 (↑26%)	0.0117* ±0.0024 (↑27%)	1.36* ±0.17 (↑40%)	-

[a] Data were obtained from page 91 (Table 15-2), page 93 (Table 16-2) and pages 259-260, 263-264 (Raw Data) of the study report.
*Significantly different from the control ($p \leq 0.05$).
** Significantly different from the control ($p \leq 0.01$).

[b] Relative weight to body weight (%) at scheduled kill after 78 weeks of treatment.

TABLE 7. Comparison of significant organ and terminal body weight changes to controls

	Males			Females		
Dose level (ppm NNI-0101):	**60**	**250**	**1000**	**60**	**250**	**1000**
	Ratio of mean weight to control value (%)					
Body weight	104	94	87*	111	104	80**
Organ						
Brain						
Absolute wt	99	94**	93**	106**	101	99
Relative wt	96	100	107	100	98	125**
Liver						
Absolute wt	98	87	130	111	124	134**
Relative wt	94	93	151*	101	120	168**
Heart						
Relative wt	100	107	116*	100	109	126**
Thyroids						
Relative wt	97	93	125	78	102	127*
Kidneys						
Relative wt	104	102	111	102	111	140**
Epididymides						
Absolute wt	100	85	73**	-	-	-

[a] Data were obtained from page 28 (Text Table 4), 259-260, and 263-264 (Raw Data) of the study report.
*Significantly different from the control ($p \leq 0.05$).
** Significantly different from the control ($p \leq 0.01$).

2. **Gross pathology:** In evaluating macroscopic changes, three groups/sex/dose level were evaluated for statistical significance: all examined animals in the dose group (ALL), animals killed as scheduled after 78 weeks of treatment (TERM), and animals that were killed *in extremis* or were found dead during the course of the study (KD). The percentage per incidence for each group with statistically significant effects are calculated and listed in the corresponding table.

Among the more common changes observed was hair loss. In males, significant increased incidences of hair (fur) loss was found in males in the 250 ppm (ALL, KD) and 1000 ppm (ALL) dose groups; however, the number of incidences did not correlate with the dose. In females, significant hair loss was observed at 1000 ppm (ALL, TERM). In females hair loss was seen more frequently in the head and dorsal regions; there were no specific areas where hair loss was more frequent in males. A significant increase in incidences of loss of tactile hair also was seen in males (ALL, TERM) and females (ALL) in the 1000 ppm dose group. Significant increases in incidences of soiled fur in the abdominal and external genital regions also were found in males in the 1000 ppm group found dead or killed *in extremis* (KD).

In the liver of males in the 1000 ppm dose group, there was a significant increase in incidences of dark color (ALL, TERM), coarse surface (ALL, TERM), and spots (ALL). No significant changes in the liver were observed in females. Males in the 1000 ppm group showed a significant increase in spots (ALL, TERM, KD) and masses (ALL, TERM) in the testis, and significant decreases in hypertrophy of the seminal vesicle (ALL, TERM) and coagulating glands (ALL, TERM).

In the kidneys of males in the 1000 ppm group, there was a significant increase in pelvic dilatation

(ALL, KD), and significant decreases in the incidences of cysts (ALL, TERM) in females and enlargement (KD) in controls. Females in the 250 ppm dose group had an increase in incidences of coarse surface of the kidney (ALL). Females in the 1000 ppm group showed a significant increase in incidences of thymic enlargement (TERM).

Other statistically significant changes in macroscopic lesions included: decreased incidences of lung masses (males, 60 ppm, TERM), increased incidences of lung masses (females, 60 ppm, ALL), decreased incidence of scabs on the skin (males, 250 ppm, ALL), and increased incidences of masses of the uterus (female, 250 ppm, TERM). There was no dose response for any of these measurements.

The statistically significant changes in incidences of macroscopic lesions are summarized in Table 8.

TABLE 8. Statistically significant changes in incidences of macroscopic lesions[a]

		Number of findings							
		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (con)**	**60**	**250**	**1000**	**0 (con)**	**60**	**250**	**1000**
Organ/finding	**Group[b]**								
Systemic/external appearance									
Loss of tactile hair	ALL	0	0	4	12**(23%)	11(21%)	7	13	21*(40%)
	TERM	0	0	1	10**(19%)	9	4	9	16
Soiled fur (abdominal)	KD	2(4%)	2	5	9*(17%)	0	0	0	0
Soiled fur (external genital region)	KD	5(9%)	6	12	14*(27%)	1	3	3	1
Skin									
Fur loss	ALL	2	4	11**(21%)	8*(15%)	11(21%)	17	12	30**(58%)
	TERM	2	3	5	4	8(15%)	10	9	26**(50%)
	KD	0	1	6*	4	3	7	3	4
Scab	ALL	8	5	2*	6	0	0	0	2
Liver									
Dark in color	ALL	0	0	0	9**(17%)	0	0	0	0
	TERM	0	0	0	7**(13%)	0	0	0	0
Spot(s)	ALL	2(4%)	2	4	8*(15%)	2	6	2	2
Coarse surface	ALL	0	0	0	6*(12%)	0	2	0	3
	TERM	0	0	0	5*(10%)	0	0	0	3
Thymus									
Enlargement	TERM	1	2	0	0	2(4%)	3	1	8*(15%)
Lung									
Mass(es)	ALL	13	6	10	9	3	11*	4	9
	TERM	11	4*	8	7	2	6	3	7
Kidney									
Coarse surface	ALL	1	2	1	2	1	1	7*	1
Cyst(s)	ALL	11(21%)	9	6	1**(2%)	4	4	1	5
	TERM	9(17%)	8	5	1**(2%)	4	3	1	5
Pelvic dilatation	ALL	8(15%)	7	8	18*(35%)	1	0	0	0
	KD	1(2%)	4	2	11**(21%)	0	0	0	0
Enlargement	KD	4(8%)	1	2	0*	0	0	0	1
Testis									
Spot(s)	ALL	2(4%)	1	1	14**(27%)	-	-	-	-
	TERM	2(4%)	1	0	8*(15%)	-	-	-	-
	KD	0	0	1	6*(12%)	-	-	-	-

TABLE 8. Statistically significant changes in incidences of macroscopic lesions[a]

		Number of findings							
		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (con)**	**60**	**250**	**1000**	**0 (con)**	**60**	**250**	**1000**
Organ/finding	**Group[b]**								
Mass(es)	ALL	0	1	0	6*(12%)	-	-	-	-
	TERM	0	1	0	4*(8%)	-	-	-	-
Seminal vesicle									
Hypertrophy	ALL	15 (33%)	16	10	3**(6%)	-	-	-	-
	TERM	15 (33%)	14	10	2**(4%)	-	-	-	-
Coagulating gland									
Hypertrophy	ALL	17	16	11	3**(6%)	-	-	-	-
	TERM	16	14	10	2**(4%)	-	-	-	-
Uterus									
Mass(es)	TERM	-	-	-	-	1	4	9**	1

[a] Data were obtained from pages 26 and 27 (Text Table 3), and 266-535 (Raw Data) of the study report.
*Significantly different from the control ($p \leq 0.05$) using Fisher's exact test.
** Significantly different from the control ($p \leq 0.01$) using Fisher's exact test.
[b] ALL = All animals examined (n= 51, 52, 52, 52 for males and 52, 52, 52, 52 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively).
TERM = Animals killed as scheduled after 78 weeks of treatment (n=34, 33, 32, 32 for males and 42, 38, 38, and 43 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively).
KD = Animals killed *in extremis* or found dead (n=17, 19, 20, 20 for males and 10, 14, 14, 9 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively).

3. Microscopic pathology: As for macroscopic changes, in evaluating microscopic changes, three groups/sex/dose level were evaluated for statistical significance: all examined animals in the dose group (ALL), animals killed as scheduled after 78 weeks of treatment (TERM), and animals that were killed *in extremis* or were found dead during the course of the study (KD). The percentage per incidence for each group with statistically significant effects are calculated and listed in the corresponding table.

a) Non-neoplastic

There were no statistically significant changes in microscopic findings in either sex in the 60 ppm dose group.

Several significant changes were observed in the liver of animals in the 1000 ppm dose group. Significant increases in incidences of centrilobular hepatocellular hypertrophy (ALL, TERM, KD) and single cell hepatocyte necrosis (ALL, TERM) were noted in both sexes. A significant increase in incidences of focal necrosis of hepatocytes was found in males (ALL, TERM, KD). Also in the 1000 ppm dose group, there was a significant decrease in incidences of microgranuloma in females (ALL). Significant decreases in incidences of centrilobular hepatocellular fatty changes were noted in males in both the 250 ppm and 1000 ppm (ALL) dose groups.

A significant increase in incidences of follicular cell hypertrophy in the thyroid was noted in both

sexes in the 1000 ppm dose group (ALL, TERM). This finding also was seen in 3 males (2 TERM, 1 KD) of the 250 ppm group (not statistically significant).

A significant increase in incidences of intracytoplasmic eosinophilic bodies in mucosal epithelial cells of the nasal cavity was found in both sexes in the 1000 ppm dose group. A significant increase in eosinophilic bodies in respiratory cells was seen in both sexes (ALL, TERM); a significant increase in eosinophilic bodies in olfactory cells was seen in males (ALL, TERM, KD).

A significant increase in incidences of diffuse acinar cell atrophy of the pancreas was seen in females of the 1000 ppm dose group (ALL, TERM). The lesion was distributed throughout the pancreas, in contrast to spontaneous focal acinar cell hypertrophy in this strain of mouse.

In males in the 1000 ppm dose group, there was a significant increase in incidences of interstitial cell hyperplasia of the testis (ALL, TERM) (17%); this finding also was seen in 3 males in the KD group. Other than the 1000 ppm dose group, interstitial cell hyperplasia was seen only in one animal in the 60 ppm dose group (TERM). Also observed in the 1000 ppm group was a significant increase in incidences of atrophy of seminiferous tubules (KD) (23% vs. 8% in the control). At 1000 ppm, a significant decrease in incidences of retention of secreted material was noted in males in the seminal vesicle and coagulating glands (ALL, TERM).

In females in the 250 ppm (TERM) and 1000 ppm (ALL, TERM) groups, there was a significant increase in incidences of endometrial hyperplasia of the uterine horn. A significant decrease in incidences of adenomyosis in the uterine horn was seen in females (ALL, TERM) in the 1000 ppm dose group. Females in the 1000 ppm group also showed a significant increase in incidences of glandular epithelial cell hyperplasia of the mammary gland (ALL, TERM).

Other statistically significant changes included a significant increase in incidences of subcapsular cell hyperplasia in the adrenal gland of males in the 250 and 1000 ppm treatment groups (ALL, TERM), a significant decrease in incidences of osteoarthrosis in the knee joint of females in the 1000 ppm dose group (ALL, TERM), and a significant decrease in incidences of cortical cysts in the kidney in males in the 1000 ppm dose group (ALL, TERM).

Statistically significant changes in incidences of non-neoplastic lesions are summarized in Table 9.

TABLE 9. Statistically significant changes in incidences of non-neoplastic lesions[a]

		Number of findings							
		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (con)**	**60**	**250**	**1000**	**0 (con)**	**60**	**250**	**1000**
Organ/finding	**Group[b]**								
Joint (knee)									
Osteoarthrosis	ALL	37	17 (19a) - (0b)	16 (20a) - (0b)	33	35 (67%)	5 (14a) - (0b)	5 (14a) - (0b)	22** (42%)
	TERM	27			20	31 (60%)			20** (38%)

TABLE 9. Statistically significant changes in incidences of non-neoplastic lesions[a]

		Number of findings							
		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (con)**	**60**	**250**	**1000**	**0 (con)**	**60**	**250**	**1000**
Organ/finding	**Group[b]**								
Nasal cavity									
Intracytoplasmic eosinophilic body, respiratory epithelial cell	ALL	2 (4%)	7	3	19** (37%)	12	12	6	23* (44%)
	TERM	2 (4%)	6	2	15** (29%)	11	11	4	21* (40%)
Intracytoplasmic eosinophilc body, olfactory epithelial cell	ALL	5 (9%)	3	7	28** (54%)	11	8	7	18
	TERM	3 (6%)	2	3	19** (37%)	10	6	5	16
	KD	2 (4%)	1	4	9* (17%)	1	2	2	2
Liver									
Fatty change, hepatocyte, centrilobular	ALL	8 (15%)	9	1* (2%)	1* (2%)	1	1	0	0
Necrosis, hepatocyte, focal	ALL	1	0	1	18**(34%)	0	4	4	0
	TERM	1	0	1	10**(19%)	0	2	2	0
	KD	0	0	0	8**(15%)	0	2	2	0
Necrosis, hepatocyte, single cell	ALL	9	7	8	26**(50%)	2(4%)	2	3	32**(61%)
	TERM	7	5	7	18**(34%)	2(4%)	2	3	31**(60%)
Hypertrophy, hepatocyte, centrilobular	ALL	0	0	0	42**(81%)	0	0	0	46**(88%)
	TERM	0	0	0	29**(56)	0	0	0	42**(81%)
	KD	0	0	0	13**(25%)	0	0	0	4*(8%)
Microgranuloma	ALL	3	1	1	0	5	5	1	0*
Kidney									
Cyst, cortical	ALL	19	10 (31 a)	6 (31a)	2**(4)	4	4 (17 a)	1 (15a)	6
	TERM	15	8 (12 b)	5 (11b)	1**(2%)	4	3 (3b)	1 (1b)	6
Thyroid									
Hypertrophy, follicular cell	ALL	0	0	3	11** (21%)	0	0	0	22** (42%)
	TERM	0	0	2	8** (15%)	0	0	0	21** (40%)
Adrenal									
Hyperplasia, subcapsular cell	ALL	18 (35%)	21	28* (54%)	32** (62%)	37	6 (14 a)	6 (16a)	39
	TERM	14 (27%)	18	22* (42%)	22* (42%)	33	- (0b)	0 (2b)	32
Pancreas									

TABLE 9. Statistically significant changes in incidences of non-neoplastic lesions[a]

		Number of findings							
		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (con)**	**60**	**250**	**1000**	**0 (con)**	**60**	**250**	**1000**
Organ/finding	**Group[b]**								
Atrophy, acinar cell, diffuse	ALL	0	0 (19a) - (0b)	0 (20a) - (0b)	0	0	0	0	8** (15%)
	TERM	0			0	0	0	0	8** (15%)
Testes									
Hyperplasia, interstitial cell	ALL	0	1	0	9** (17%)	-	-	-	-
	TERM	0	1	0	6**				
Atrophy, seminiferous tubule	KD	4 (8%)	5	5	12* (23%)	-	-	-	-
Seminal vesicle									
Retention of secreted material	ALL	20 (39%)	19	12	4** (8%)	-	-	-	-
	TERM	19 (37%)	15	11	3** (6%)	-	-	-	-
Coagulating gland									
Retention of secreted material	ALL	18 (35%)	13	11	4** (8%)	-	-	-	-
	TERM	16 (31%)	11	9	2** (4%)	-	-	-	-
Uterine horn									
Hyperplasia, endometrium	ALL	-	-	-	-	23 (44%)	24	31	33* (63%)
	TERM	-	-	-	-	20 (38%)	23	27* (52%)	30* (58%)
Adenomyosis	ALL	-	-	-	-	8 (15%)	6	6	1* (2%)
	TERM	-	-	-	-	8 (15%)	6	4	1* (2%)
Mammary gland									
Hyperplasia, glandular epithelial cell	ALL	-	-	-	-	5(9%)	6	11	19** (37%)
	TERM	-	-	-	-	3	5	8	15** (29%)

[a] Data were obtained from pages 31 and 32 (Text Table 6), 266 and 535 (Raw Data) of the study report.
*Significantly different from the control ($p \leq 0.05$) using Fisher's exact test.
** Significantly different from the control ($p \leq 0.01$) using Fisher's exact test.
[b] ALL = All animals examined (n= 51, 52, 52, 52 for males and 52, 52, 52, 52 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively, except where indicated in parentheses).
TERM = Animals killed as scheduled after 78 weeks of treatment (n=34, 33, 32, 32 for males and 42, 38, 38, and 43 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively, except where indicated in parentheses).
KD = Animals killed *in extremis* or found dead (n=17, 19, 20, 20 for males and 10, 14, 14, 9 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively).

Values for "n" in parenthesis designated by "a" indicates parameter was examined on animals that showed macroscopic lesions at terminal kill and on all animals killed *in extremis* or found dead during the study; not statistically analyzed. Values for "n" in parenthesis designated by "b" indicates parameter was examined in animals that showed macroscopic lesions; not statistically analyzed.

Incidences of skin lesions at sites other than the lumbodorsal region defined by the study protocol,

referred to as "other sites" were not subjected to statistical analysis because the limited number of animals examined was limited, and there were individual variations on sites and numbers examined among animals. However, the incidences of dermatitis in females in the 1000 ppm group (ALL, TERM) were more notably frequent than that of the control. At terminal kill, most of the dermatitis at "other sites" corresponded to macroscopic hair loss. Dermatitis in lesions with hair loss was slight and consisted of inflammatory cell infiltration to the dermis in the absence of epidermal abnormalities. Eight cases of dermatitis observed in females in the 1000 ppm group (TERM) were found in the head, compared with the control group in which 1 of 2 incidences of dermatitis occurred in the head with hair loss.

The incidences of dermatitis at "other sites" are summarized in Table 10.

TABLE 10. Incidences of dermatitis ("other"sites)[a]

		Number of findings[b] (n)							
		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (control)**	**60**	**250**	**1000**	**0 (control)**	**60**	**250**	**1000**
Organ/finding	**Group[c]**								
Skin (other)									
Dermatitis	ALL	12 (16)	10 (14)	12 (22)	10 (18)	3 (13)	4 (21)	6 (15)	11 (29)
	TERM	8 (11)	5 (7)	5 (10)	3 (9)	2 (9)	2 (13)	3 (10)	10 (24)
	KD	4 (5)	5 (7)	7 (12)	7 (9)	1 (4)	2 (8)	3 (5)	1 (5)

[a] Data were obtained from page 34 (Text Table 7), and pages 266-535 (Raw Data) of the study report. "Other" refers to lesions at sites other than the lumbodorsal region.

[b] Examined on animals with macroscopic lesions; not statistically analyzed.

[c] ALL = All animals examined.
TERM = Animals killed as scheduled after 78 weeks of treatment.
KD = Animals killed *in extremis* or found dead.

b) Neoplastic

A statistically significant increase in incidences of interstitial cell tumors of the testis (12/52, 23%) was found in males in the 1000 ppm dose group (ALL, TERM, KD). The tumor in one male found dead at Week 64 was thought likely to have caused death because the tumor was as large as 35 mm in diameter, macroscopically. No clear characteristics of malignancy (i.e., metastasis or invasion through the capsule) were seen in interstitial tumors of any animals in the high-dose group. In one male in the 60 ppm dose group, the interstitial cell tumor infiltrated to the epididymis. However, because no animals in the 250 ppm group had tumors or preneoplastic hyperplasia of the interstitial cells, the malignant interstitial cell tumor seen in the 60 ppm group was considered to be a spontaneous lesion.

Other significant changes in incidences of neoplasms included decreases in malignant pulmonary adenomas in males (ALL) and in malignant lymphomas in females (ALL) in the 1000 ppm dose group.

Organs and tissues which developed neoplastic lesion were similar in the test substance treatment

groups when compared with the control. There was no increase in rare tumors in the treated groups when compared to IET control data.

Statistically significant changes in incidences of neoplastic lesions are summarized in Table 11. The total number of benign and malignant tumors found in all study groups is summarized in Table 12.

TABLE 11. Statistically significant changes in incidences of neoplastic lesions[a]

		Number of findings							
		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (cont.)**	**60**	**250**	**1000**	**0 (cont.)**	**60**	**250**	**1000**
Organ/lesion	**Group[b]**								
Systemic tumor									
Lymphoma (malignant)	ALL	6	6	4	3	9	7	3	1**
Lung									
Adenocarcinoma (malignant)	ALL	8	2 (24a)	3 (30a)	2*	2	6 (20a)	1 (19a)	5
Testes									
Interstitial cell tumor (benign)	ALL	0	0	0	12** (23%)	-	-	-	-
	TERM	0	0	0	7** (13%)	-	-	-	-
	KD	0	0	0	5** (10%)	-	-	-	-

*Significantly different from the control ($p \leq 0.05$) using Fisher's exact test.
** Significantly different from the control ($p \leq 0.01$) using Fisher's exact test.
[a] Data were obtained from pages 29 and 30 (Text Table 5) of the study report.
[b] ALL = All animals examined (n= 51, 52, 52, 52 for males and 52, 52, 52, 52 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively, except where indicated in parentheses).
TERM = Animals killed as scheduled after 78 weeks of treatment (n=34, 33, 32, 32 for males and 42, 38, 38, and 43 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively).
KD = Animals killed *in extremis* or found dead (n=17, 19, 20, 20 for males and 10, 14, 14, 9 for females in the 0, 60, 250 and 1000 ppm dose groups, respectively).

Values for "n" in parenthesis designated by "a" indicates parameter was examined on animals that showed macroscopic lesions at terminal kill and on all animals killed *in extremis* or found dead during the study; not statistically analyzed.

TABLE 12. Number of benign and/or malignant neoplasms[a]

		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (control)**	**60**	**250**	**1000**	**0 (control)**	**60**	**250**	**1000**
Parameter	**Group[b]**								
No. of benign neoplasms	ALL	34	19	24	38	13	6	8	15
	TERM	27	14	16	28	12	3	8	13
	KD	7	5	8	10	1	3	0	2
No. of malignant neoplasms	ALL	26	14	15	13	22	21	20	15
	TERM	18	7	7	8	11	10	10	10
	KD	8	7	8	5	11	11	10	5
No. of benign and malignant neoplasms	ALL	60	33	39	51	35	27	28	30
	TERM	45	21	23	36	23	13	18	23
	KD	15	12	16	15	12	14	10	7
No of animals with benign									

TABLE 12. Number of benign and/or malignant neoplasms[a]

		Males				Females			
Concentration of NNI-0101 in diet (ppm):		**0 (control)**	**60**	**250**	**1000**	**0 (control)**	**60**	**250**	**1000**
Parameter	**Group[b]**								
neoplasms	ALL	26	17	17	29	13	6	8	15
	TERM	19	12	11	21	12	3	8	13
	KD	7	5	6	8	1	3	0	2
No. of animal with malignant neoplasms	ALL	26	13	14	12	19	19	15	12
	TERM	18	7	6	7	10	9	8	8
	KD	8	6	8	5	9	10	7	4
No. of animals with neoplasms	ALL	37	25	26	34	28	22	21	25
	TERM	25	16	15	22	19	11	14	19
	KD	12	9	11	12	9	11	7	6

[a] Data were obtained from pages 95 (Table 17-2, end), 96 (Table 17-3, end), 98 (Table 17-5, end), 100 (Table 18-2, end), 102 (Table 18-4, end) , 104 (Table 18-6, end), and pages 266-535 (Raw Data) of the study report.
*Significantly different from the control ($p \leq 0.05$).
** Significantly different from the control ($p \leq 0.01$).
[b] ALL = All animals examined
TERM = Animals killed as scheduled after 78 weeks of treatment
KD = Animals killed *in extremis* or found dead

Values for "n" in parenthesis designated by "a" indicates parameter was examined on animals that showed macroscopic lesions at terminal kill and on all animals killed *in extremis* or found dead during the study; not statistically analyzed.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

In order to evaluate the carcinogenic potential of NNI-0101 technical in mice, the test substance was administered in feed to SPF ICR (Crj:DC-1) mice of both sexes at a dose level of 0, 60, 250, or 1000 ppm for a period of 18 months (78 weeks). Each dose group consisted of 52 males and 52 females. During the treatment period, all animals were checked daily for mortality and general condition. Body weight and food consumption were also monitored once a week from 1 to 13 and every 4 weeks thereafter. After 78 weeks of treatment, all surviving animals were subjected to hematological examinations. Organ weight analysis was performed on 10 animals/sex/sgroup. All animals were subjected to necropsy. Histopathological examination was performed on systemic organs and tissues from all animals killed in extremis and found dead during the study, systemic organs and tissues from all animals subjected to terminal kill in the control and 1000 ppm groups, and the target organs in animals subjected to terminal kill in 60 and 250 ppm groups. The treatment realted changes are summarized below:

1000 ppm group: In general for clinical observations, significant increases and/or increasing trends were noted in incidence of loss of tactile hair in both sexes and loss of fur in females. Body weights in both sexes were significantly lower than the controls throughout the treatment period and mean body weights at termination of treatment in males and females were 89% and 74% of those of the controls, respectively. Organ weight measurement revealed that significant increases or increasing trends in the absolute and relative weights of the liver and relative weight of the thyroids in males and females. Males also exhibited a significant decrease in the absolute epididymide weight, and females had a loss in absolute kidney weight. In addition to the loss of hair, necropsy revealed an increases incidene of dark liver, spot (s) and coarse surface in the liver, and spot (s) and masses in the testis were observed in males. For neoplastic neoplastic histopathological lesions, a significant increase in incidence of interstitial cell tumor in the testes was noted in males. For non-neoplastic histopathological lesions, significant increases in incidence of centrilobular hepatocellular hypertrophy and single cell necrosis of hepatocyte were noted in males and females. In addition, a significant increase in incidence of focal hepatocellular necrosis was found in males. A significant increase in incidence of follicular cell hypertrophy in the thyroid was noted in males and females. For the nasal, a significant increase in incidence of intracytoplasmic eosinophilic body in respiratory epithelial cell was also observed in males. Furthermore, significant increases in increases in incidence of interstitial cell hyperplasia and atrophy of the seminiferous tubule in the testis and subscapular cell hyperplasia in the adrenal were noted in males. Significant increases in incidence of diffuse acinar cell atrophy of hyperplasia in the adrenal were noted in males. Significant increases in incidence of diffuse acinar cell atrophy of the pancreas, glandular epithelial cell hyperplasia in the mammary gland, and endometrial hyperplasia in the uterine horn were noted in females. Females also showed an increasing tendancy in incidence of dermatitis in the hair loss lesions.

250 ppm group: In general clinical observation, a significant increase in incidence of loss of tactile hair was noted in males. In males, although mean body weight at termination of treatment was comparable to the control, it was continuously low during the early phase of the treatment period. In histopathological examination, a significant increase in incidence of subscapular cell hyperplasia in the adrenal was noted in males. Follicular cell hypertrophy in the thyroid was observed 3/52 males. A significant increase in incidence of endomentrial hyperplasia in the uterine horn was noted in

females.

60 ppm group: No treatment-related changes were observed in either sex.

As shown from the data, the incidence of interstitial cell tumor in the testis was increased in males treated at 1000 ppm when NNI-0101 Technical was repeatedly dosed orally to SPF ICR (Crj-CD-1) mice for 78 weeks. The change was, however considered not to suggest the direct carcinogenic action of the test substance and likely to be a secondary change to anti-androgenic potential of the test substance. The no-observed-adverse-effect level (NOAEL) was determined to be 60 ppm (males 6.25 mg/kg/day; females, 5.82 mg/kg/day) under the conditions of the present study.

B. REVIEWER COMMENTS:

The purpose of this study was to evaluate the carcinogenic potentiatal of pyrifluquinazon in mice for 18 months through oral exposure. Male and female mice were fed pyrifluquinazon in their diets in the following concentrations; 0, 60, 250 and 1000 ppm (equivalent to 0, 6.25/ 5.82, 27.1/ 25.0, and 122/ 120 mg/kg/day [M/F]). Body weights, and food consumption were measured throughout the study. At 78 weeks, the animals were sacrificed and toxicity was observed in all parameters except for extensive hematological examination, and clinical chemical parameters. Under these conditions, the results were as follows:

At 1000 ppm, there was an increased incidence of tactile hair loss in males (23% vs. 0% in the control), and there was an increasing trend of this sign in females (40% vs. 21% in the control). A significant increase in incidences of fur loss in females was suspected of being treatment-related (58% vs. 21% in the control).

Mean body weights of males in the 1000 ppm group were significantly lower than the control at Week 2 (↓4%), and from Week 4 to the end of the treatment period (↓5-17%). Females in the 1000 ppm group had significantly lower mean body weights at Weeks 4 and 5 (↓5%), and from Week 7 to the end of the treatment period (↓7-26%).

At termination of treatment at 1000 ppm, the mean body weights of males and females in the 1000 ppm group were 89% and 74% of the control values, respectively. A non-statistically significant increase in mean relative liver weight was found in males (↑30%), and in females there was a significant increase in both mean absolute and relative liver weights (↑34% and ↑67%). Finally there was a significant increase in relative weight of kidneys in females (↑40%).

At 1000 ppm, there was a significant increase in incidences of seminiferous tubule atrophy in males that were killed *in extremis* or found dead. There was also a significant decrease in mean absolute epididymide weights observed in males which was thought to be related to the testicular lesions (↓28%). There was a significant increase in incidences of mammary gland hyperplasia in females (37% v. 9% in the control), and a significant increase in incidences of endometrial hyperplasia of the uterine horn of females (63% vs. 44% in controls). Statistically significant changes associated with the liver were increases in incidences of centrilobular hypertrophy and cell necrosis of hepatocytes in both sexes (81% for males and 88% in females vs. 0% in the controls), and increases of incidences of focal necrosis of hepatocytes (50-61% for both sexes) and dark-colored liver (17% vs. 0% in the control), coarse surface (12% vs. 0% in the control), and spots seen at necropsy in males (15% vs. 0%

in the control).

At 1000 ppm, there were significant increases in incidences of follicular cell hypertrophy of the thyroid in both sexes (21% in males and 42% in females vs. 0% in the controls). A significant increase in incidences of subcapsular cell hyperplasia of the adrenals was observed in males (62% vs. 35% in the control). Increased incidences of diffuse acinar cell atrophy in the pancreas were noted in females (15% vs. 0% in the control). Significant increases in incidences of intracytoplasmic eosinophilic bodies in respiratory epithelial cells (29-40% for both sexes vs. 4-21% in the controls) and in olfactory cells (males, 54% vs. 9% in controls) were observed.

Finally at the 1000 ppm concentration, there were significant increased incidences of benign interstitial (leydig) cell tumors (23% vs. 0 in the control), interstitial (leydig) cell hyperplasia (17% vs. 0 in the control), and masses (12 % vs.0 in the control) in the testes in males. Tumor increases were considered to be a secondary effect resulting from anti-androgenic activity of the test substance and not a result of a direct carcinogenic property of the test substance.

At the 250 ppm concentration, there was a persistent depression in mean body weight in males from Week 8 to 56 (↓5-8%). Slight increases in incidences of follicular cell hypertrophy of the thyroid were seen in some males. There were small incidences of tactile hair loss in males. There was also a significant increase in incidences of endometrial hyperplasia of the uterine horn in females (52% vs. 44% in the control). Finally, there was a significant increase in incidence of subcapsular cell hyperplasia of the adrenals in males (54% vs. 35% in the control).

There were no treatment related effects at the 60 ppm concentration.

The Reviewer is in agreement with the investigator's conclusions. There were no apparent deficiencies in study methods. This mouse carcinogenicity study is consistent with the results from the rat carcinogenicity study in that there are increased interstitial (leydig) cell tumors in male testes. There are also decreased epidymide weights and other testicular effects, as well as evidence of hyperplasia in the uterine tissues of females. These effects are consistent with the registrant's proposed anti-androgen receptor mode of action as described in the CARC document for pyrifluquinazon (TXR# 0056339). Furthermore, it was the opinion of the CARC that the tumors in mice were treatment related, while the tumors in the rat were not treatment related due to a high background incidence in the control. Of particular significance among the mode of action studies submitted by the registrant, is the mouse hormone study (MRID 48707901), where evidence is provided showing that pyrifluquinazon does in fact cause elevated levels of luteinizing hormone (LH) at 70.7 and 136.2 mg/kg/day. Elevated levels of dihydrotestosterone (DHT) were demonstrated at 136.2 mg/kg/day. These increased hormone levels are consistent with the results in a similar study in rats (MRID 48306981D).

Other organs worth noting which were affected in this study were the liver, kidney and nasal cavity.

The systemic LOAEL for mice is 250 ppm (equivalent to 27.1 mg/kg/day for males, 25.0 mg/kg/day for females), based on decreased mean body weight in males; and increased incidences of tactile hair loss in males, endometrial hyperplasia of the uterine horn in females, follicular cell hypertrophy of the thyroid in males, and subcapsular cell hyperplasia of the adrenal in males. The NOAEL is 60 ppm (equivalent to 6.25 mg/kg/day for males and 5.82

mg/kg/day for females).

At the doses tested there was a treatment-related increase in the incidence of benign interstitial (leydig) cell tumors in males in the 1000 ppm treatment group when compared with the control. Dosing was considered adequate. There was evidence of toxicity without an increase in morality was seen at the highest tested dose (1000 ppm), which was selected based on a 90-day study.

C. **STUDY DEFICIENCIES:** No deficiencies were noted.

DATA EVALUATION RECORD
Pyrifluquinazon
PC Code: 555555
TXR#: 0055820
MRID#: 48306966

Study Type: Combined Chronic Toxicity / Carcinogenicity Study in Rats
OPPTS 870.4300 [§83-5];

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Tetrahedron Incorporated
1414 Key Highway, Suite B
Baltimore, MD 21230

Principal Reviewer Henry Spencer, Ph.D. Date 7/24/11

Secondary Reviewer Nasrin Begum, Ph.D. Date 7/25/11

Tetrahedron Program Manager Nasrin Begum, Ph.D. Date 7/25/11

Quality Control Daniel Ewald, B.S. Date 7-25-11

Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-47
EPA Reviewer//WAM: Dunbar// Brunsman/Farwell

Disclaimer

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Anwar Dunbar, Ph.D. **Signature**: _____
Risk Assessment Branch I, Health Effects Division (7509P) **Date:** _____
EPA Reviewer: Chester Rodriguez, Ph.D. **Signature:** _____
Risk Assessment Branch I, Health Effects Division (7509P) **Date:** _____

Template version 02/06

TXR #: 0055820

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity study in rats (dietary); OPPTS 870.4200 [§83-5]; OECD 453.

PC CODE: 555555 **DP BARCODE:** D387307

TEST MATERIAL (PURITY): Pyrifluquinazon (98% a.i.)

SYNONYMS: NNI-0101 Technical, Pyrifluquinazon, R-40598 Technical grade, 1-acetyl-3,4- dihydro-3-[(3-pyridinylmethyl) amino]-6-[1,2,2,2-tetrafluro-1-(trifluoromethyl)ethyl-2(1H)-quinazolinone

CITATION: Kuwuhara, Maki. (2006) NNI-0101 Technical: Carcinogenicity Study in Rats. Testing Facility: The Institute of Environmental Toxicology, 4321 Uchimoriya-machi, Joso-shi, Ibaraki, 303-0043, Japan. Laboratory Project No.T-29016, September 28, 2006. (MRID 48306966 Unpublished.)

SPONSOR: Nihon Nohyaku Co., Ltd. 2-5 Nihonbashi 1-Chime, Chuo-ku, Tokyo 103-8236, Japan

EXECUTIVE SUMMARY:

In a carcinogenicity study (MRID 48306966), Pyrifluquinazon (98% a.i.; Lot/Batch No. EFZ0013G) was administered in the diet to SPF (Fischer 344/DuCr) rats (50/sex/dose) for up to 104 weeks at concentrations of 0, 100, 350, 1300 ppm (equivalent to 0/0, 3.53 / 4.51, 12.5 / 16.4, 48.5 / 60.2 mg/kg bw/day [M/F]). Changes in clinical condition, and changes in body weights and food consumption were determined, as well as macro and microscopic tissue observations were performed. Clinical chemical parameters, urine analysis and most hematological measurements were not tested in this study. Only the WBC and differentials were performed and discounted due to abnormal blast cells and abnormal lymphocytes present.

There were no treatment-related effects in the 100 ppm group. Rats at 100 ppm did not differ from controls in regards to body weights or food intake throughout the study.

At 350 ppm there were numerous effects on clinical signs, body weights, as well as macro- and microscopic changes that were considered treatment-related and adverse. Regarding clinical signs, there was an increased incidence of eye opacity in males (18% vs. 8% in the control). Both males and females lost body weight starting from week 72 to the end of the study (4-7% in males), and from week 32 to the end of the study (3-10% in females). Epididymide weights were reduced (↓35%). Gross examination found a reduction in testes softening (24%) compared to controls (45%), with a small increase in the incidence of benign tumor masses (100%) compared to controls (86%). Epididymide softening was significantly elevated (97%) when compared to controls (77%). Microscopic evaluations of non-neoplastic lesions in males at 104 weeks of treatment, showed reductions in mammary gland cysts (9.7%) compared to controls (34%). Males also exhibited increased eye opacity (20%) vs. the controls (9%). Based on the statistical evaluation of the male tumor incidences, the conclusion is that the chemical in the

diet for 104 weeks induces additional testicular interstitial cell tumors in the treated male rats at 350 ppm of pyrifluquinazon (98% vs. 82% in the controls).

At 1300 ppm there were similar but more robust treatment-related adverse effects observed. For clinical signs, there was an increased incidence of eye opacity for both sexes (68% in males and 94% in females vs. 8-10% in the controls). There was also an increase in the tail mass of males (20% vs. 6% in the controls) and an increase in the length of incisors in females (14% vs. 2%). There were other seemingly treatment related effects whose biological significance is not well understood such as a decreases in read adhesive substance in females (12% vs. 48% in the controls, and a decrease in skin calluses in males (0% vs. 8% in controls).

At 1300 ppm, there were decreased body weights in both sexes from week 64 to the end of the study in males (↓4-20%), and from week 32 to the end of the study in females (↓3-20%). Absolute organ weights at necropsy were reduced in the males for brains (↓4%), and epididymides (↓55%). Organs with increased statistically significant weight values were the liver (↑22%) and kidneys (↑11%). Absolute organ weights in females which were statistically significantly reduced were the brain (↓6%), spleen (↓73%) and adrenals (↓23%). Gross examination found reduction in testes softening compared to controls (46% vs. 7%), with small increase in the incidence of benign tumor masses in the 1300 ppm group (97% vs. 85% in the controls). Epididymide softening was significantly elevated (97%) when compared to controls (77%). Significant opacity of the eyes was noted at both sexes (78%) and (97%) compared to controls in males and females respectively (9% and 8%). For animals carried to termination at 104 weeks, pituitary masses were significantly reduced (7%) in males at when compared to controls (45%). Female macroscopic examination revealed changes including decreases in eye discharge (2% vs. 24% in the controls), decreased mammary gland hypertrophy (0% vs. 16% in the control), spleen enlargement (0% vs. 13% in the control), and decreased thyroid masses (0% vs. 13% in the controls).

For males at 1300 ppm, mammary gland cysts were reduced (0% vs. 34% in controls), increases were reported in striated muscle atrophy (90% vs. 11% in controls), nasal cavity rhinitis (61% vs. 34% in controls), liver centrilobular hypertrophy (93% vs. 0% in controls), chronic nephropathy of kidneys (73% vs. 25% in controls), atrophy of epididymides (100% vs. 14% in controls), seminal vesicle atrophy (97% vs. 0% in controls), coagulating gland atrophy (97% vs. 0%), prostate atrophy (93% vs. 0% in controls), thyroid increased small-sized follicles (93% vs. 0% in controls), cataract of eye (100% vs. 9% in controls), retinal atrophy (97% vs. 9% in controls) and adrenal hypertrophy of the zona fasciculata/ reticularis cells (2% vs. 0% in controls). Smaller numbers of positive occurrences were exhibited in those found dead and killed in extremis for the following fatty changes in liver, liver cell hypertrophy, increased # small-sized follicles of thyroid, hypertrophy of adrenal zona cells, and cataract of the eye.

Neoplastic lesions were examined microscopically in animals at 104 weeks of treatment and males exhibited an increase in mammary gland fibroadenomas of 5 vs. 1 in controls. Thyroid C-cell adenomas were reduced to 6 vs. 16 in controls and testicular interstitial cell tumors were increased to 41 vs. 33 in controls. When the additional animals were found dead are added, the totals become: thyroid C-cell adenomas 6 **/ 20 when compared to controls. The various individual tumor types (all benign) were not increased in females, but were significantly reduced in incidences: pituitary gland anterior adenoma (4** vs. 20 in controls), mononuclear leukemia (1** vs. 9 in controls and 1** vs.9 in controls) in both the 350 and 1300 ppm groups, and thyroid gland C-cell adenoma (3** vs. 13 in the controls). The biological significance of the decreases in these incidences due to treatment is unclear.

Testicular interstitial (leydig) cell tumors had an increased incidence of 98% and 94% vs. 82% in controls at 350 ppm and 1300 ppm. There was one male at the high dose level that was cannibalized and lost to analysis for this parameter.

The extensive changes in the reproductive organs of the males, suggests an anti-androgenic effect of the chemical. The mode of action cannot be discerned from the data available in this study. The male tumor incidences in the top two doses exceeded the historical control data submitted with the study. Based on

the statistical evaluation of the male tumor incidences, the conclusion is that the chemical in the diet for 104 weeks induces additional tumors in the treated male rats at 350 and 1300 ppm of pyrifluquinazon.

The LOAEL is 350 ppm (equivalent to12.5 / 16.4 mg/kg/day in males/females), based upon decreased body weights in both sexes. In males, there was a decrease in epydidymide weights, increased epididymide softening, decreased testes softening and increased testes mass, increased incidences of testicular atrophy, seminal vesicle atrophy, coagulating gland atrophy, prostate atrophy, and an increased incidence of eye cataracts and interstitial tumors in the testes. In females there was bile duct hyperplasia, decreased zymogen granules and focal acinar cell atrophy in the pancreas, tubular basophilic changes in the kidney, dilation of the uterine horn and retinal atrophy, and spleen hematopoeisis. The NOAEL is 100 ppm (equivalent to 3.53/ 4.51 mg/kg/day in males/females).

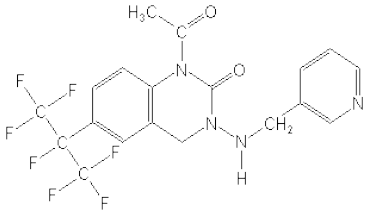
This study is classified as **acceptable/guideline** as a carcinogenicity study in rats (OPPTS 870.4200; OECD 451).

COMPLIANCE: Signed and dated GLP Compliance, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0101 (Pyrifluquinazon)

Description:	Pale yellow powder
Lot/Batch No.:	EFZ0013G
Purity (w/w):	98% a.i.
Stability of compound:	The Sponsor stated that the compound was stable until September 20, 2007 in the diet stored in the animal room at ambient air for 5 days.
CAS #:	337458-27-2
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	(SPF) Fischer (F344/DuCr)
Age and group mean weight at study initiation:	Approximately 5 weeks old 86-101 g males; 76-90 g females
Source:	Atsugi Breeding Center, Charles River, Japan
Housing:	By sex in groups of 5 on study, unless reduced by mortality or isolation. The cages were suspended, stainless steel and wire mesh.
Diet:	Certified diet MF Mash from the Oriental Yeast Co., Ltd., Tokyo, Japan., *ad libitum*,
Water:	Filtered and hypochlorite and UV light sterilized well water, *ad libitum*
Environmental conditions	Animal room No. 102
Temperature:	23 ± 2ºC
Humidity:	55 ± 15%
Air changes:	10 +/h
Photoperiod:	12 hours light/12 hours dark (7 am -7 pm)
Acclimation period:	9-10days

B. STUDY DESIGN

1. In life dates: Start: October 16, 2003 End: Males: October 14, 17, 18, 2005
Females: October 24, 25, 2005

2. Animal assignment: Animals were randomly assigned, stratified by body weight, to the test groups presented in Table 1. Animals used were within ± 20% of a group mean body weight for the sex.

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg/day; M/F)	Main study 24 months (# rats/sex)
Control	0	0/0	50
Low (LDT)	100	3.53/ 4.51	50
Mid (MDT)	350	12.5/ 16.4	50
High (HDT)	1300	48.5/ 60.2	50

a Data were obtained from text-table on page 16 of MRID 48306966.

3. **Dose-selection rationale:** Dose-selection was based on the results of a subchronic oral toxicity study (IET 02-0068), where Pyrifluquinazon was administered in the diet to Fischer F344 rats (10/sex/dose) at doses of 0, 50, 100, 500, or 2500 ppm for 90 days. The assumed (unstated) LOAEL was 500 ppm in males/females), based on increased liver weights, increased reticulocyte counts and increased plasma lipids in females. The assumed (unstated) NOAEL was 100 ppm in males/females).

4. **Treatment preparation, analysis, and administration:** The test substance was incorporated as a pre-mix into the diet to provide the required dietary concentrations. No adjustment for purity was made. The test substance formulations were prepared to cover the dietary requirements over 2 weekly periods. When not in use, the diet formulations were stored at room temperature.
The Sponsor stated that stability of the test substance in the diet has been demonstrated in a previous study (IET 02-5019) where samples of 10 and 4,000 ppm were analyzed after a sealed storage at 21-25°C for 35 days followed by exposure to ambient air and room temperature 14 to 21 days. (data not reported in this MRID). This time period covered the period of storage and usage for this study. The homogeneity and concentrations of the test substance in diet was to be verified at each concentration on the first formulations and approximate at 3, 6, 9 12, 15, and 18 months of the study.

   Results:

   Homogeneity (% coefficient of variation): Reported as 2.2% or lower for the doses of 100, 350, and 1300 ppm respectively.

   Stability (% of initial): 93%- 98% of initial vales found after use of the diet, and appears to be stable for the period of mixing, storage and use.

   Concentration (% of nominal): 93-98% of nominal concentration

Dose (ppm)	Mean ± S.D.
0	-
100	98.6 ± 0.72
350	350.9 ± 6.12
1300	1304.0 ± 21.79

N=5

   The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** The following analyses were conducted and significance was denoted at the 5% and 1% levels. (methods are extracted from pp. 23 of the study report.) The data on body weight, food consumption, total leukocyte count, differential leukocyte count and organ weights were evaluated by Bartlett's' test for equality of variance between the control and treated groups. When group variances were homogeneous, the one-way layout ANOVA was conducted to determine if any statistical differences exist among groups. When the analysis of variance was significant, Dunnett's multiple comparison tests were applied. When the group variances were heterogeneous, the data was evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant, Dunnett type mean rank sum test was applied.

Life table analysis was used for the data of mortality. Final mortality including the animals killed in extremis or found dead during the period of terminal kill was not subjected to the statistical analysis.

Fischer's exact probability test (one-tailed analysis) was used to analyze the data on general clinical signs and incidence of macroscopic and histopathological findings. Clinical signs observed during the period of terminal kill were included in the data for the statistical analysis. Gross and histopathological lesions of the animals killed in extremis or found dead during the terminal kill analyzed together with dose which died during the treatment period.

C. METHODS

1. Observations

a. Cage side observations: Animals were checked for morbidity and mortality at least once daily in the mornings. All animals were observed for clinical signs at least once daily.

b. Clinical examinations: Detailed physical examinations including palpation for masses were performed at least weekly. Clinical signs observed were recorded by sign, date of onset, severity, and duration. Observations would take place for reactions of the animals in their home cage; observations of the animal's reactions to handling and the observations of the physical conditions of the animals. Finally, observations were made of the animals with their reactions in the open field as behavioral changes.

2. Body weight: Each animal was weighed prior to treatment, weekly for the first 13 weeks of study, approximately every 4 weeks from week 16 to week 104 prior to necropsy.

3. Food consumption and compound intake: Food consumption (g/rat/day) was recorded for a period of 4 consecutive days in a week for the first 13 weeks and every 4 weeks from week 16 to the end of the study. Compound intake (mg/kg/day) was calculated based on nominal concentration, group mean food consumption, and group mean body weight values.

4. Ophthalmoscopic examination: During the acclimatization phase, all animals were examined for ophthalmic problems. All surviving animals were re-examined after approximately 24 months of treatment.

5. Hematology and clinical chemistry: Blood was sampled from ether anesthetized animals by puncture of the posterior vena cava after overnight dietary fasting. Blood analyses for WBC and differentials were performed on all the surviving animals and when possible, a blood smear was prepared for moribund animals, just before sacrifice. Blood smears were taken after 52 weeks, 78 weeks, and after 104 weeks for all animals. * Blood smears were only analyzed when other hematology parameters were abnormal (when 0.3 X 10^3/µl or more of large unstained cells (LUC) were counted on the hematology analyzer). The following CHECKED (X) parameters were examined.

a. Hematology: Only total WBC and differential evaluation of the blood smears were done when the above *criterion was met.

X	Leukocyte count (WBC)*	X	Leukocyte differential count*

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

b. Clinical chemistry:
Clinical chemistry analyses were not performed on animals of this study.

6. Urinalysis: Urinalysis was not performed on animals of this study.

7. Sacrifice and pathology: Necropsy was performed on all animals found dead during treatment as well as those killed in extremis, and those to be sacrificed at study end. There were no interim sacrifices in this study. The animals were sacrificed under deep ether anesthesia by exsanguination from the abdominal aorta and the posterior vena cava after being fasted overnight. Necropsy of all animals included the examination of the whole body and recording of all macroscopic findings.

The following organs or tissues were sampled (X) and weighed (XX) at necropsy:

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	XX	Thymus		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	XX	Thyroids*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum)
	Bile duct* (rat)	XX	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
		XX	Ovaries*+	X	Harderian gland
	RESPIRATORY	XX	Uterus*+ (with cervix)	X	Articular surface (knee joint)
X	Trachea*	X	Mammary gland*	X	All gross lesions and masses*
X	Lung*++	X	Vagina	X	Vertebrae
X	Nose* [a]			X	Head
X	Pharynx*			X	Coagulating glands
X	Larynx*				

* Recommended for combined chronic toxicity/carcinogenicity studies based on Guideline 870.4300
\+ Organ weight required in combined chronic toxicity/carcinogenicity studies
++ Organ weight required if inhalation route

Histopathology:

Tissue and organ samples were fixed by immersion in neutral buffered 10% formalin1. Samples were routinely processed and stained with hematoxylin and eosin. Histopathology examinations of the above samples were performed for: 1) animals in the 0 and 1300 ppm groups, 2) animals found dead during the treatment period in the 100 and 350 ppm groups, 3) the liver, kidney, adrenal, thyroid, eye and mammary gland from both sexes; the pituitary, testis, epididymis, prostate, seminal vesicle, coagulating gland, skeletal muscle, nasal cavity from males, and the pancreas, ovary, uterine horn, uterine cervix and vagina from females, and gross lesions from all animals killed at termination in the 100 and 350 ppm groups.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity:

Table 1. Clinical signs of Toxicity

Summary of clinical observations:	Sex and dose level (ppm)							
	Males				Females			
Lesions and signs:	**0**	**100**	**350**	**1300**	**0**	**100**	**350**	**1300**
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Behavior: Decreased spontaneous motor activity	6	5	6	8	11	5	3↓	5
Respiration: Bradypnea	6	5	5	8	10	5	3↓	5
Skin: Red adhesive substance	3	4	4	6	24 (48%)	19 (38%)	17 (34%)	6↓↓ (12%)
Mass	23	22	21	23	17	11	14	8
Fur: Loss of fur	5	2	0	5	12	15	18	17
Soiled fur	6	2	4	3	5	4	4	0
Eye/eyelid: Opacity	4 (8%)	4 (8%)	9 (18%)	34↑↑ (68%)	5 (10%)	2 (4%)	6 (12%)	47↑↑ (94%)
Mouth: Elongation of incisor	1	0	0	2	1 (2%)	1 (2%)	2 (4%)	7↑ (14%)
Extremities: Callus	8	6	4	0↓↓	0	0	0	0
Tail: Mass	3 (6%)	8 (16%)	4 (8%)	10↑ (20%)	0	1 (2%)	0	0

Data extracted from page 25, and pages 178-212 (Raw Data) of study report
↓, ↑ $p<0.05$ (By Fischer's exact probability test)
↑↑, ↓↓ $p<0.01$
Percentage incidence was not calculated.

The incidences of treatment-related signs of toxicity observed at 350 ppm and below were not increased compared to controls in both sexes, except for eye/eyelid opacity. A statistical decrease in the numbers of animals with a loss of fur however did occur. There were increased numbers of male animals with tail masses (20%) at 1300 ppm. Significant changes in the amount of red adhesive substance occurring was reported in females at 1300 ppm (12% vs 48% in the control). Treatment related significant increased numbers of animals (males and females vs. controls respectively) occurred at 1300 ppm with eye/lid problems or opacity of the eyes (68% for males and 94% for females vs. 8-18% in the controls). This increase also took place at 350 ppm in males (18% vs. 8% in the control). Female rats at 1300 ppm showed an increase in the numbers with the elongation of the incisors (14% vs 2% in the control). Males exhibited fewer calluses at the highest dose. Females showed a decrease in the spontaneous motor activity at 350 ppm, but there were no significant changes at both higher and lower doses.

2. Mortality: Mortality rates were reported for weeks of 90 – 94, and were significantly decreased for females in the 1300 ppm group. This change is not considered to be treatment related. No significant mortality changes occurred in the males of this group.

Survival rates in the other treated groups were similar to controls. Mortality figures in males were 15/50, 8/50, 9/50, and 9/50 and in females were 12/50, 7/50, 6/50, and 5/50 in the 0, 100, 350, and 1300 ppm groups respectively. One additional female in the 0 ppm group died at the terminal sacrifice.

B. <u>BODY WEIGHT AND BODY WEIGHT GAINS</u>: Males appeared to be more resistant to body weight changes until much later in the study. The top 2 dose groups (350, 1300 ppm) started statistically significant reductions in weights at about week 64 to 72 and continued lower to the end of the 104 week study. Reductions showed a decline from 97% to 94% and from 96% to 80% in the 350 and 1300 ppm groups respectively. Only sporadic reductions in weights in males at 100 ppm were seen and were not consistent. The values are a percentage of the control values for each sex. Statistical significances given by Dunnett's multiple comparison test and were reported as $p<0.05$, $p<0.01$ in the study report, pp 26. See Table 2.

Increased body weights were generally noted in the first weeks starting at week 3 until week 16 in the female rats in the 1300 ppm group (↑3-6%). At week 32, both the 350 and 1300 ppm groups of females showed a decreased body weight compared to controls. Body weights of the females in the 100 ppm group showed a slight decline in body weight of about 1-7% of controls which achieved statistical significance only at week 52. The 350 ppm females exhibited weight reductions from 2-10% compared to controls from week 36 to termination. The 1300 ppm group females also exhibited reductions in body weights that generally declined from 97% to 78% of the control values. See Table 3.

Table 2. Body weight – Group mean value in male rats (g)

Dose (ppm)		Week													
		3	**11**	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**	**104**
0	Mean	187	314	345	380	404	422	435	441	450	459	458	456	451	447
	S.D.	9	14	16	19	21	23	24	25	27	27	27	31	32	31
	N	50	50	50	50	50	49	49	49	49	48	48	46	42	35
100	Mean	187	311	340	373	395 * (↓3%)	412	424	429	437	440 * (↓5%)	444 * (↓4%)	447	438	426 ** (↓5%)
	S.D.	8	13	16	18	20	21	23	24	25	39	29	31	34	33
	N	50	50	50	50	50	50	50	50	50	49	48	44	42	42
350	Mean	188	311	341	374	398	415	427	433	440	444 * (↓4%)	436 ** (↓5%)	437 ** (↓5%)	427 ** (↓5%)	419 ** (↓7%)
	S.D.	7	12	14	16	18	19	22	22	23	24	25	27	31	31
	N	50	50	50	50	50	50	50	50	50	50	50	48	45	41
1300	Mean	188	316	346	380	404	419	429	435	435 * (↓4%)	426 ** (↓8%)	409 ** (↓11%)	396 ** (↓14%)	382 ** (↓16%)	361 ** (↓20%)
	S.D.	8	13	14	16	17	18	19	17	18	20	20	21	27	23
	N	50	50	50	50	50	50	50	49	49	49	47	47	45	41

Data were obtained from Tables 5-1, 5-2, 5-3 on pages 73-75, and pages 213-236 (Raw Data) of the Study report
S.D.: standard deviation
N: Number of animals
Significant difference from control: * p <= 0.05; ** p <= 0.01

Table 3. Body weight – Group mean value in female rats (g)

Dose (ppm)		Week													
		3	**11**	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**	**104**
0	Mean	127	169	179	188	196	204	214	222	231	244	248	255	262	260
	S.D.	5	8	10	11	12	14	17	18	22	42	28	26	21	29
	N	50	50	50	50	50	50	50	49	49	48	47	46	42	38
100	Mean	126	167	177	185	194	201	208	215	221	229	236	245	250	257
	S.D.	4	8	9	10	11	12	13	13	15	18	21	25	24	22
	N	50	50	50	50	50	50	50	50	50	48	48	48	47	43
350	Mean	128	169	178	184	192 * (↓3%)	197 ** (↓4%)	204 ** (↓5%)	210 ** (↓6%)	218 ** (↓6%)	221 ** (↓10%)	225 ** (↓10%)	231 ** (↓10%)	238 ** (↓10%)	242 * (↓7%)
	S.D.	5	9	9	9	9	9	9	10	14	15	17	19	19	19
	N	50	50	50	50	50	50	50	50	50	49	48	48	46	44

1300	Mean	131 ** (↑3%)	179 ** (↑6%)	186 ** (↑6%)	190	194	198 ** (↓3%)	201 ** (↓7%)	204 ** (↓9%)	206 ** (↓11%)	208 ** (↓15%)	210 ** (↓16%)	212 ** (↓17%)	212 ** (↓20%)	209 ** (↓20%)
	S.D.	5	7	7	7	7	7	7	8	8	8	9	10	12	9
	N	50	50	50	50	50	50	50	50	50	50	50	50	49	45

Data were obtained from Tables 6-1, 6-2, 6-3 on pages 76-78 of Study report, and pages 237-260 (Raw Data) of the Study report
S.D.: standard deviation
N: Number of animals
Significant difference from control: * p <= 0.05; ** p <= 0.01

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** As presented in Table 8-3, pp 84 of the study report effects on food consumption were pretty much restricted to the high dose group females during the study with only an occasional 4 week time period that was statistically significantly lower/ increased from that of the controls. The 1300 ppm group however, exhibited statistically significant increases in food intake at week 3 and continued through the first 16 weeks of treatment (↑7%) compared to the female control group for the same period of time. After week 48 the food intake started to decline and was statistically significant for the conclusion of the study. The average food intake values for the females were: 9.4, 9.2, 9.4, and 9.0 grams/animal/day for the entire study.

   Using the same time frames for comparison, (week 3- week 16) the high dosed males consumed more food, 14.69 g/animal/day (↑7.3%) compared to 13.7 g/animal/day in the male control group.
   The 350 ppm group males displayed a slight increase in food consumption which only achieved statistical significance sparsely throughout the study when compared to the control group.
   The average food intake per group of males for the 104 week study was reported as: 13.4, 13.4, 13.6, 13.8 g/animal/day for the 0, 100, 350, and 1300 ppm groups respectively. Data were extracted from Tables 7 and 8 on pages 79-84 of the study report.

2. **Compound consumption:** The mean achieved dosages are reported in Table 9 on page 85 of the study report. See Table 4.

Table 4. Compound consumption of the treatment groups as mean average for 104 weeks (mg/kg/day)

Males		Females	
100 ppm	3.53 mg/kg/day	100 ppm	4.51 mg/kg/day
350 ppm	12.5 mg/kg/day	350 ppm	16.4 mg/kg/day
1300 ppm	48.5 mg/kg/day	1300 ppm	60.2 mg/kg/day

Data extracted from Table 3, page 28 of study report, and pages 261-272 (Raw Data), values calculated from food consumption values

D. **OPHTHALMOSCOPIC EXAMINATION:** After 104 weeks of treatment in the 350 and 1300 ppm males, increased incidences of eye lesions were reported. Opacity of the eye was noted in both males (94%) and females (68%) of the 1300 ppm group, and a slight increase was also seen at the 350 ppm in males. Control values were 10% and 8% for males and females respectively. See Table 5.

Table 5. Incidence of treatment-related ophthalmological findings noted at the 104 week examination for all animals on study.				
Females				
Dietary concentrations (ppm)	0	100	350	1300
Group size	50	50	50	50
Opacity	5 (10%)	2	6	47** (94%)
Males				
Dietary concentrations (ppm)	0	100	350	1300
Group size	50	50	50	50
Opacity	4 (8%)	4	9	34** (68%)

Data extracted from Table 1, page 25, and pages 300-599 of study report
**Statistically significant at p<0.01 by Fischer's exact probability test.

E. BLOOD ANALYSES

1. **Hematology:** Only the WBC and the cell differential were completed for a hematology requirement. The study authors state that no adverse, treatment-related effects were observed on hematological parameters. The increases noted in the WBC, lymphocyte and LUC counts were discounted and considered to be unusable due to the numbers of abnormal lymphocytes and increased numbers of blast cells found on visual slide examination. See Tables 6 and 7.

Table 6. Hematology – Group mean values in male rats after 104 weeks of treatment									
Dose (ppm)	**No. of animals examined**	**WBC (10^3/ µL)**		**Differential leukocyte count (10^3/ µL)**					
				L	**N**	**M**	**E**	**B**	**LUC**
0	35	Mean	6.57	3.21	2.88	0.31	0.10	0.02	0.05
		S.D.	1.81	0.81	1.24	0.10	0.03	0.03	0.05
100	42	Mean	8.91	3.31	2.80	0.33	0.09	0.02	0.48
		S.D.	16.43	0.97 (41)	1.55	0.18	0.04	0.02 (41)	2.75
350	41	Mean	8.71	2.94	2.82	0.33	0.10	0.02	0.41
		S.D.	17.17	0.76 (40)	1.68	0.29	0.04	0.01 (40)	2.35
1300	41	Mean	22.79	2.62 ** (↓19%)	6.29	0.66	0.06 ** (↓40%)	0.02 *	6.06 * (↑12020%)
		S.D.	66.39	0.76 (38)	13.78	1.75	0.02	0.01 (38)	21.96

Data were obtained from Table 11 on page 88 of study report, and pages 273-281 (Raw Data) of study report
S.D.: Standard deviation
(): Available number of animals for the parameter.
Significantly different from control: *, p <= 0.05; **, p <= 0.01
WBC: Total leukocyte count
L: Lymphocyte
N: Neutrophil
M: Monocyte
E: Eosinophil
B: Basophil
LUG: Large unstained cell

Table 7. Hematology – Group mean values in female rats after 104 weeks of treatment									
Dose (ppm)	**No. of animals examined**	**WBC (10^3/ µL)**		**Differential leukocyte count (10^3/ µL)**					
				L	**N**	**M**	**E**	**B**	**LUC**
0	37	Mean	8.58	3.46	2.47	0.25	0.06	0.04	0.89
		S.D.	17.63 (36)	4.21 (35)	3.71 (36)	0.26 (36)	0.03 (36)	0.06 (35)	4.66 (36)
100	43	Mean	5.26	3.54	1.34	0.18	0.05	0.04	0.10
		S.D.	5.24 (42)	4.61 (42)	0.45 (42)	0.13 (42)	0.02 (42)	0.13 (42)	0.25 (42)

350	44	Mean	3.71	2.01	1.44	0.15 * (↓40%)	0.05	0.02	0.04 * (↓96%)
		S.D.	1.31 (43)	0.79 (43)	0.72 (43)	0.06 (43)	0.02 (43)	0.01 (43)	0.03 (43)
1300	45	Mean	4.15	2.24	1.61	0.16 * (↓36%)	0.09	0.01 ** (↓75%)	0.04 * (↓96%)
		S.D.	1.46	0.76	0.64	0.06	0.25	0.01	0.02

Data were obtained from Table 12 on page 89, and pages 283-290 (Raw Data) of Study report
S.D.: Standard deviation
(): Available number of animals for the parameter.
Significantly different from control: *, p <= 0.05; **, p <= 0.01
WBC: Total leukocyte count
L: Lymphocyte
N: Neutrophil
M: Monocyte
E: Eosinophil
B: Basophil
LUG: Large unstained cell

F. URINALYSIS: Urinary analysis was not performed in this study.

G. Clinical Chemistry: Clinical Chemistry values were not determined in this study.

SACRIFICE AND PATHOLOGY

1. Organ weights: After 104 weeks of treatment, a decreased mean body weight was noted in the 100 ppm males (↓10%), and also decreased in the 1300 ppm males (↓23%) when compared to controls. The increase in liver weight was 2.2 grams greater than controls (↑22%) at 1300 ppm. The absolute weight of the brain was slightly reduced compared to the controls (↓4%). Kidney weights were slightly increased (↑11%). The epididymides were significantly reduced in weights at both the 350 ppm (↓35%) and 1300 ppm (↓55%) dose levels compared to controls. See Table 8.

Absolute organ weights in females in the 1300 ppm groups were decreased in the brain (↓6%), and spleen (↓73%). Relative weights for the brain, heart, and kidneys were increased at the level and the liver at level for those 10 animals evaluated per group after 104 weeks of treatment. See Table 9.

Table 8. Organ weight – Group mean values in male rats
Absolute weight at terminal kill after 104 weeks of treatment

MALES	Organ weights - absolute			
	0	**100**	**350**	**1300**
Body weight at termination(g)	433± 23	393 ± 40 * (↓10%)	401 ± 20	334 ± 30 ** (↓23%)
Brain (mg)	2087 ± 41	2078 ± 35	2091 ± 56	2006 ± 59 * (↓4%)
Thyroids (mg)	28 ± 10.5	41.5 ± 59.9	24.3 ± 5.8	28.7 ± 13.4
Heart (mg)	1152 ± 116	1114 ± 93	1103 ± 47	1096 ± 67
Liver (g)	10.19 ± 0.71	9.92 ± 1.20	11.02 ± 1.18	12.40 ± 1.9 ** (↑22%)
Kidneys (mg)	2538 ± 168	2589 ± 485	2581 ± 199	2824 ± 216 * (↑11%)
Spleen (mg)	975 ± 125	980 ± 212	1193 ± 787	3116 ± 4791
Adrenals (mg)	75.8 ± 59.1	53.7 ± 7.1	54.4 ± 3.6	63.4 ± 10.6
Testes (mg)	3408 ± 987	3770 ± 1311	3798 ± 662	3532 ± 1369
Epididymides (mg)	598 ± 96	512 ± 164	392 ± 52 * (↓35%)	270 ± 74 ** (↓55%)

[a]Data were obtained from Table 15-1, page 105, and pages 292-293 (Raw Data) of study report
*Significantly different (p<0.05) from the control group
**Significantly different (p<0.01) from the control group

Table 9. Organ weight – Group mean values in female rats
Absolute weight at terminal kill after 104 weeks of treatment

Dose (ppm)		Body weight (g)	Brain (mg)	Thyroids (mg)	Heart (mg)	Liver (g)	Kidneys (mg)	Spleen (mg)	Adrenals (mg)	Ovaries (mg)	Uterus (mg)
0	Mean	249	1869	30.3	779	6.42	1641	1626	57.6	147.4	1388
	S.D.	30	45	43.4	58	0.92	66	3316	6.6	281.1	933
	N	10	10	10	10	10	10	10	10	10	10
100	Mean	242	1866	13.7	738	6.23	1573	2015	242.8	62.0	1062
	S.D.	24	36	2.7	38	1.22	149	4581	602.2	19.7	283
	N	10	10	10	10	10	10	10	10	10	10
350	Mean	230	1861	14.9	778	6.01	1605	526	49.8	70.7	1117
	S.D.	16	36	2.5	39	0.44	56	117	6.8	24.9	270
	N	10	10	10	10	10	10	10	10	10	10
1300	Mean	196 ** (↓22%)	1774 ** (↓6%)	15.5	762	6.22	1632	446 ** (↓73%)	44.4 ** (↓23%)	47.3	1237
	S.D.	9	28	1.5	31	0.62	143	37	6.0	16.0	557
	N	10	10	10	10	10	10	10	10	10	10

Data were obtained from Table 16-1, page 107 of the study report, and pages 296-297 (Raw Data), of the study report
S.D.: Standard deviation.
N: Number of animals.
Significantly different from control: *, p<= 0.05; **, p <=0.01.

Table 10. Organ weight – Group mean values in female rats
Relative weight to body weight (%) at terminal kill after 104 weeks of treatment

Dose (ppm)		Brain	Thyroids	Heart	Liver	Kidneys	Spleen	Adrenals	Ovaries	Uterus
0	Mean	0.76	0.0121	0.32	2.61	0.67	0.72	0.023	0.062	0.54
	S.D.	0.09	0.0169	0.04	0.48	0.09	1.55	0.005	0.121	0.32
	N	10	10	10	10	10	10	10	10	10
100	Mean	0.78	0.0056	0.31	2.58	0.65	0.86	0.102	0.026	0.44
	S.D.	0.07	0.0007	0.04	0.50	0.06	1.99	0.254	0.008	0.13
	N	10	10	10	10	10	10	10	10	10
350	Mean	0.81	0.0065	0.34	2.61	0.70	0.23	0.022	0.031	0.49
	S.D.	0.06	0.0010	0.02	0.10	0.05	0.05	0.003	0.009	0.14
	N	10	10	10	10	10	10	10	10	10
1300	Mean	0.91 ** (↑20%)	0.0079	0.39 ** (↑22%)	3.18 * (↑22%)	0.83 ** (↑23%)	0.23	0.023	0.024	0.64
	S.D.	0.03	0.0006	0.01	0.36	0.09	0.02	0.004	0.008	0.30
	N	10	10	10	10	10	10	10	10	10

Data were obtained from Table 16-2 on page 108, and pages 298-299 (Raw Data) of the study report
S.D.: Standard deviation.
N: Number of animals.
Significantly different from control: *, p<= 0.05; **, p <=0.01.

Gross pathology: In male animals necropsied at the termination of the study and those found dead or killed in extremis, macroscopic changes were noted particularly at the top two dose levels. There were statistically significant and reductions in the numbers of animals exhibiting a softening of the testes at both the 350 (24%) and 1300 ppm (7%) groups compared to controls respectively (46%). At the same time increases in numbers of testicular masses were also seen with the 350 ppm group (100%) reaching statistical significance as compared to controls (85%). The epididymides exhibited softening with numbers increased to 40 in each of the top two dose levels (both 97%) compared to 27 animals in the controls (77%). See Table 11.

Eyes showed increases of opacity in both the top two doses and was statistically significantly increased (78%) to 32 animals compared to but 3 animals in controls (9%). See Table 11.

Skin appeared to lose the ability to form calluses at 1300 ppm males, though the change was not statistically significant. See Table 11.

Effects noted macroscopically in animals that were found dead or were killed in extremis included a reduction in the livers showing a coarse surface. Eye opacity was not significantly increased in these few males. See Table 12.

The incidences of other findings in the treated groups were similar to the control groups or without histological correlate.

Table 11. Macroscopic changes of selected organs in males at terminal Kill after 104 weeks

Dietary concentrations **Group size**	**0 ppm** **N=35**	**100 ppm** **N=42**	**350 ppm** **N=41**	**1300 ppm** **N=41**
Spleen enlarged	0	4	3	3
Testes softening	16 (46%)	19	10 * (24%)	3 ** (7%)
mass	30 (85%)	37	41 * (100%)	40 (97%)
Epididymides softening	27 (77%)	33	40 ** (97%)	40 ** (↑97%)
Thyroid spots	6	4	6	0 *
Eye opacity	3 (9%)	4	8	32 ** (78%)
Skin callosity	33	5	2	0

Data extracted from Tables 13-1 and 13-2 on pages 90-91, and pages 300-457 (Raw Data) of study report
*p<0.05
**p<0.01

Table 12. Macroscopic changes in selected organs in males killed in extremis, or found dead on study

Dietary concentrations **Group size**	**0 ppm** **N=15**	**100 ppm** **N=8**	**350\ ppm** **N=9**	**1300 ppm** **N=9**
Liver coarse surface	6	2	2	0 *
Testes softening	4	4	5	4
masses	6	1	6	7 * (77%)
Seminal vesicle atrophy	2	1	0	1
Eye opacity	1	0	1	2
Thoracic cavity masses	0	3 * (37.5%)	0	0

Data were extracted from Tables 13-3 through Table 13-5 on pages 90-94., and pages 300-457 (Raw Data) of the study report
*p<0.05 Statistically significantly different from controls.

In necropsied female rats at terminal sacrifice, several changes were noted macroscopically. Eye discharges were reduced in the high dose group (2% vs. 24% in the control). Mammary gland hypertrophy was also reduced (0% vs. 16% in the control) at the 1300 ppm dose level. At 1300 ppm several other changes were noted as decreased when compared to control values. These included spleen enlargement in the high dose group (0% vs. 13% in the control), decreased coarse surface of the liver (0 vs. 11% in the control), decreased pituitary masses in the high dose group (7% vs. 45% in the control), and finally decreased thyroid masses in the high dose group (0% vs. 14% in the control group. Increased incidences were also noted as uterine luminal dilatation treated in the high dose group (19/45) vs. 6/37 in controls, Eye ball opacity in the high dose group (97% vs. 8% in the control group). Animals that were found dead or killed in extremis exhibited statistically significant reductions in eye discharge at the 1300 ppm dose (0% vs. 77% in the control). Finally, skin masses were also reduced (0% vs. 31% in the control). See Table 13.

Table 13. Macroscopic changes in selected organs of females after 104 weeks on study

Dietary concentrations **Group size**	**0 ppm** **N=37**	**100 ppm** **N=43**	**350 ppm** **N=44**	**1300 ppm** **N=45**
Eye discharge	9 (24%)	12	9	1 ** (2%)
Mammary gland hypertrophy	6 (16/%)	5	6	0**

Spleen enlargement	5 (13%)	4	1	0*
Liver coarse surface	4 (11%)	2	1	0 *
Uterus luminal dilatation	6 (16%)	7	12	19 (42%)
Pituitary masses	17 (45%)	8 ** (18%)	14	3 ** (7%)
Thyroid masses	5 (14%)	4	1	0 *
Eyeball opacity	3 (8%)	2	4	44 ** (97%)
Skin masses	10	10	8	8

*p<0.05
**p<0.01 statistical significance from controls

Table 14. Macroscopic changes in selected organs of females found dead or killed in extremis on study.

Dietary concentrations **Group size**	**0 ppm** **N= 13**	**100 ppm** **N=7**	**350 ppm** **N=6**	**1300 ppm** **N=5**
Skin masses	4 (31%)	3	3	0
Eye discharge	10 (77%)	4	5	0 **

Data extracted from Table 14-3 on pages 100 and 101, and pages 458-599 (Raw Data) of the study report
**p<0.01 statistical significance from control

3. Microscopic pathology

a. Non-neoplastic: At the 104 week termination, there was an increased incidence of numerous microscopic findings in the 1300 ppm dose in the males. The percentages of these findings/changes are calculated as the incidence number divided by the total animals in that particular group. Changes included striated muscle atrophy (90% vs. 11% in control at 1300 ppm), nasal cavity rhinitis (61% vs. 34%) in controls, liver centrilobular hepatocyte hypertrophy (93% vs. 0% in controls), epididymis atrophy (100% vs. 14% in control), seminal vesicles atrophy (97% vs. 0% in control), coagulating gland (98% vs. 0% in control), prostate atrophy (93% vs 0% in control), thyroid increased small-sized follicles (93% vs. 0% in control), thyroid follicular cell hypertrophy (61% vs. 0% in controls), adrenal zone fasciculate/reticularis cells hypertrophy (32% vs. 0% in control), eye cataract (100% vs. 9% in controls), and finally eye retinal atrophy (97% vs. 9% in control). See Table 15.

Effects occuring in to the 350 ppm dose group of males included a lowered occurrence of mammary gland cysts (9.7% vs. 34% in the controls), and seminiferous tubule atrophy (19% vs. 31% in control). See Table 15.

Increased effects at 350 ppm included epididymide atrophy (73% vs. 14% in control), seminal vesicle atrophy (41% vs. 0% in control), coagulating gland atrophy (41% vs. 0% in control). Prostate atrophy (14.6% vs. 0% in the control) and eye cataract with (27% vs. 9% in control) were at the level of significance. See Table 15.

Table 15. Microscopic findings of non-neoplastic lesions in selected Tissues and organs of males at termination at 104 weeks.

Dietary concentrations **Group size**	**0 ppm** **N=35**	**100 ppm** **N=42**	**350 ppm** **N=41**	**1300 ppm** **N=41**
Mammary gland cyst	12 (34%)	10	4 ** (9.7%)	0**
Bone Marrow sternum	4	-	-	11
Bone Marrow femur	4	-	-	11
Muscle striated atrophy	4 (11%)	4	5	37** (90%)
Nasal cavity rhinitis	12 (34%)	9	14	25 * (61%)
Liver hypertrophy, hepatocyte, centrilobular	0	0	0	38** (93%)
Kidney tubular , basophilic change	23 (65%)	23	26	11 ** (27%)
Kidneys nephropathy chronic	9 (25%)	8	10	30 ** (73%)
Testes atrophy seminiferous tubule	11 (31%)	21	8	2 ** (18%)

Epididymide atrophy	5 (14%)	6	30 ** (73%)	41 ** (100%)
Seminal vesicle atrophy	0	3	17 ** (41%)	40 ** (97%)
Coagulating gland atrophy	0	3	17 ** (41%)	40 ** (97%)
Prostate atrophy	0	0	6 * (14.6%)	38 ** (93%)
Thyroid increased small-sized follicles	0	0	0	38** (93%)
Thyroid hypertrophy follicular cell	0	0	0	25** (61%)
Adrenal hypertrophy zone faciculata/reticularis cell	0	0	0	13** (32%)
Eye Cataract	3 (9%)	5	11 * (↑27%)	41 ** (100%)
Eye atrophy retina	3 (9%)	6	8	40** (97%)

Findings in the tissues and organs with those males were found dead or killed in extremis mirrored the finding of those reported at 104 week with the exception that only increases were found and the changes were limited to the 1300 ppm dose level. See Table 16.

Table 16. Selected tissues and organs with microscopic non-neoplastic lesions in males killed in extremis or found dead.

Dietary concentrations **Group size**	**0 ppm** **N= 15**	**100 ppm** **N= 8**	**350 ppm** **N = 9**	**1300 ppm** **N = 9**
Spleen hematopoiesis increased	2	3	2	5* (55%)
Muscle- triceps surae atrophy	0	1	1	7** (77%)
Epididymis atrophy	0	0	0	3* (33%)
Seminal vesicles atrophy	0	0	0	4** (44%)
Coagulationg gland atrophy	0	0	0	4** (44%)
Prostate atrophy	0	0	0	3* (↑33%)
Thyroid increased small sized follicles	0	0	0	5** (55%)
Eye cataract	0	0	1	4* (44%)
Eye atrophy retina	0	0	1	2

Data were extracted from Tables 19-6 through 19-9 on pages 126 – 129, and pages 300-457 (Raw Data) of the study report
*p<0.05
*p<0.01
Statistically significant from controls.

Non-neoplastic lesions in female rats found at the 104 week termination in the 1300 ppm group showed statistically significant in increased incidences of mammary gland atrophy (62% vs. 8% in control), liver pathological changes (40% for diffuse fatty changes, 100% for centrilobular fatty changes and 96% for bile duct hyperplasia vs. 0-16% for these indices in control), pathological changes of the pancreas (40-56% for indices vs. 0-16% in control), ovarian atrophy (51% vs 16% in control), uterine horn luminal dilatation (27% at 350 ppm and 64% at 1300 ppm vs. 5% in the control), with hyperplasia of the endometrial glands (22% vs. 3%in control), thyroid hyperplasia of follicles and increased numbers of small-sized follicles(93 and 88% at 1300 ppm vs. 0% in the control), hypertrophy of the zone faciculata/reticularis cells of the adrenals (100% vs 0% in the control), and cataracts and retinal atrophy (both 100% at 1300 ppm and 68% for retinal atrophy at 350 ppm vs 4-6% in the in controls). Other parameters which were elevated at included keratinization of mucosal epithelium of the vagina (13% at 1300 ppm vs. 0% in the control). See Table 17.

A reduction in the edema of the renal papillae was also noted at 1300 ppm (17% vs. the control). Several of these changes also occurred in the 350 ppm groups and included bile duct hyperplasia (75%), reduced zymogen granules of the pancreas (15% vs. 0% in the control), and an increased incidence pancreatic acinar cell atrophy at 350 ppm (31% vs. 16% in control), increased incidence of tubular basophilic change in the kidney (36% vs. 11% in controls), and retinal atrophy of the eye (68% vs. 43% in the control). See Table 17.

Table 17. Selected non-neoplastic lesions in female rats after treatment with Pyrifluquinazon in the diet for 104 weeks.

Dietary concentrations Group size		0 ppm N= 37	100 ppm N= 43	350 ppm N= 44	1300 ppm N= 45
Mammary gland	atrophy	3 (8%)	4	7	28 ** (62%)
Liver	fatty change hepatocyte, diffuse	0	0	0	40 ** (88%)
	Fatty change hepatocyte, centrilobular	0	0	0	45 ** (100%)
	Bile duct hyperplasia	16	24	33 ** (75%)	43 ** (96%)
	Foci of cellular alteration basophilic type	32	39	41	25 ** (56%)
	Microgranuloma	22	25	19	14 ** (31%)
Pancreas	vacuolation acinar cell cytoplasm	0	0	0	21 ** (47%)
	Infiltration fat	0	0	0	18 ** (40%)
	Decreased zymogen granules	0	2	7 * (15%)	35 ** (77%)
	Atrophy acinar cell focal	6 (16%)	10	14 (31%)	25 ** (56%)
	Cellular alteration, foci	0	0	0	23 ** (51%)
Kidneys	edema renal papilla	14 (38%)	18	18	8 * (17%)
	Tubular basophilic change	4	4	16 ** (36%)	14 * (31%)
Ovary	Atrophy	6 (16%)	2	1 * (2%)	23 ** (51%)
Uterine Horn	luminal dilatation, endometrial gland	2	7	12 ** (27%)	29 ** (64%)
	Hyperplasia, endometrial gland	1	3	4	10 ** (22%)
Vagina	Keratinization of mucosal epithelium	0	1	1	6* (13%)
Thyroid	increased small-sized follicles	0	0	0	42 ** (93%)
	Hypertrophy of follicular cells	0	0	0	40 ** (88%)
Adrenals	Hypertrophy. Cortical cells- focal	7	3	3	0 **
	Hypertrophy zone fasciculata/reticularis cell	0	0	0	45 ** (100%)
	Hyperplasia cortical cell focal	17	18	15	1 ** (2%)
Eye	Cataract	4	3	10	45 ** (100%)
	Retinal atrophy	6	6	30 ** (68%)	45 ** (100%)

Data were extracted from Tables 20-1 through 20-4 on pages136-139, and 458-599(Raw Data) of the study report.
Statistically significant from controls
*p<0.05
**p<0-.01

Significant changes in number of animals found dead and killed in extremis were found with changes in the liver with centrilobular hepatocyte hypertrophy (100%) and fatty changes (60%), thyroids with increased small-sized follicles (100%), adrenal zona fasiculata cells hypertrophy (100%), and increased cataracts (80%) and atrophy of the retinas (80 %) when compared to controls. See Table 18.

Table 18. Selected set of organ and tissue incidences of microscopic non-neoplastic lesions in female rats killed in extremis or found dead

Dietary concentrations Group size		0 ppm N= 13	100 ppm N= 7	350 ppm N= 6	1300 ppm N= 5
Spleen	extramedullary hematopoiesis increased	3	3	5* (83%)	4* (80%)
Liver	fatty change, centrilobular hepatocyte diffuse	0	0	1	3* (60%)
	Hypertrophy centriolobular hepatocyte	0	0	0	5** (100%)
Thyroids	Increased small-sized follicles	0	0	0	5** (100%)
Adrenals	Hypertrophy zona fascicularis/reticularis	0	0	0	5** (100%)
Eye	Cataract	1	0	1	4** (80%)
	Atrophy of retina	1	2	1	4** (80%)

Data were extracted from Tables 20-5 – 20-7 of pages 140-142, and pages 458-599 (Raw Data) of the study report.
Statistically significantly different from controls
*p<0.05
**p<0.01

Neoplastic: The incidences of neoplastic lesions in males of the treated groups of animals terminated at 104 weeks were similar to controls or were less than those of the control with the exceptions of the

mammary gland fibroadenoma, thyroid C-cell adenomas, and the testicular insterstitial cell tumors. See Table 19.

When the tumors found in the few males that had died on study or were killed in extremis were added to the 104 week termination animals, the tumor incidence for interstitial cells of the testes in the top two doses become 41/50 in controls (82%), 38/ 50 at 100 ppm 76%), 49**/50 at 350 ppm (98%) and 47*/ 49 at 1300 ppm (94%). The increases show an oncogenic effect in the top two doses. See Tables 19, 20 and 21.

Table 19. Incidences of neoplastic lesions in male rats at the 104 week termination.

Dietary concentrations **Group size**			**0 ppm N= 35**	**100 ppm N = 42**	**350 ppm N= 41**	**1300 ppm N= 41**
Mammary gland	fibroadenoma	(B)	1	2	3	5
	Adenocarcinoma	(M)	0	0	1	1
Thyroid C-cell	adenoma	(B)	16	15	18	6 ** (14%)
	carcinoma	(M)	2	0	1	0
follicular cell	adenocarcinoma	(M)	0	1	0	1
Testis	interstitial cell tumor	(B)	33 (94%)	36 (85%)	41 (100%)	41 (100%)

**p<0.01

Table 20. Incidences of neoplastic lesions in male rats that died on study or were killed in extremis

Dietary concentrations **Group size**			**0 ppm N= 15**	**100 ppm N= 8**	**350 ppm N=9**	**1300 ppm N= 9**
Testis	interstitial cell tumor	(B)	8	2	8	6
Thyroid	C-cell adenoma	(B)	4	1	1	0

Table 21. Total tumors of selected tissues in total of male rats from 104 week study.

Dietary concentrations **Group size**			**0 ppm N= 50**	**100 ppm N=50**	**350 ppm N= 50**	**1300 ppm N= 50**
Mammary gland	fibroadenoma	(B)	1	2	3	5
	Adenocarcinoma	(M)	0	0	1	1
Thyroid	C-cell adenoma	(B)	20	16	19	6* * (12%)
	C-cell carcinoma	(M)	2	0	1	0
	Follicular cell adenocarcinoma	(M)	0	1	0	1
Testis	Interstitial cell tumor	(B)	41 (82%)	38 (76%)	49** (98%)	47 * (94%)

Data were extracted from pages 300-457 of the study report.
@ The number of rats was limited to 49 for testis evaluation.
*p<0.05
**p<0.01

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: There were no treatment related increases in mortality. The eye is a target organ of the chemical, and at 1300 ppm both males and females exhibited increases in opacity of the eye. Body weight gains were considered to have occurred by the increased food consumption and of no toxicological significance. At 1300 ppm males and females showed low body weight after 64 or 36 weeks respectively. The low body weight associated with decreases in food consumption at 1300 ppm was treatment related. The depression of weight at 350 ppm in body weight was treatment related. At 100 ppm there was no treatment related effect on body weights.

Hematology WBC counts and differentials were not reliable due to the inclusion of abnormal blasts and atypical lymphocytes.

Fluctuations of weights for liver, kidney, adrenal and epididymis were treatment related. Adrenal absolute weights in the females at 1300 and 350 ppm were decreased and considered to be treatment related. Males at 1300 and 350 had increased incidences of masses in the testis and a softening of the epididymis. The uterus showed luminal dilatation in females at 1300 ppm. Other macroscopic changes were considered to be incidental.

At 350 ppm lesions were seen in animals including: Leydig cell tumor of the testis, atrophy in the epididymis and cataract in the eye of males, and bile ductal hyperplasia in the liver and retinal atrophy in the eye of females. The pancreas with vacuolization of acinar cells, had decreased zymogen granules and focal acinar cell atrophy.

Males at 1300 and 350 ppm also showed significant increases in the incidences of atrophy in the epididymides, seminal vesicle coagulating gland and prostate. At the highest dose group males and females exhibited increased amount of chronic nephropathy and/or basophilic change in the kidney. Males at 1300ppm showed significant increases of striated muscle fiber atrophy in skeletal muscle and are considered to be treatment related.

Females at 1300 ppm showed significant increases in the atrophy of the ovary, luminal dilatation of endometrial gland and endometrial hyperplasia in the uterine horn, luminal dilatation of endometrial gland in the cervix, keratinization of mucosal epithelia in the vagina and atrophy on the mammary gland. Uterine horn luminal dilatation of the endometrial gland was also seen at 350 ppm.

The incidence of Leydig cell tumors of the testis was increased in males treated at 1300 and 350 ppm of Pyrifluquinazon in the diets for 104 weeks. The changes were not considered to be suggestive of direct carcinogenic property of the test substance by likely to be secondary to its anti-androgenic potential. The NOAEL was determined to be 100 ppm (males: 3.53 mg/kg/day; females: 4.51 mg/kg/day) under the conditions of the present study.

B. REVIEWER COMMENTS

The purpose of this study was to determine the carcinogenic potential of pyrifluquinazon in rats via the dietary route of exposure at concentrations of 0, 100, 350, 1300 ppm (equivalent to 0/0, 3.53 / 4.51, 12.5 / 16.4, 48.5 / 60.2 mg/kg bw/day [M/F]) for 104 weeks. Changes in body weights and food consumption were determined, as well as macro and microscopic tissue observations were performed. Clinical chemical parameters, urine analysis and most hematological measurements were not tested in this study. Only the WBC and differentials were performed and discounted due to abnormal blast cells and abnormal lymphocytes present. Under the conditions of this study, the results are as follows:

At 1300 ppm there were similar but more robust treatment-related adverse effects observed. For clinical signs, there was an increased incidence of eye opacity for both sexes (68% in males and 94%

in females vs. 8-10% in the controls). There was also an increase in the tail mass of males (20% vs. 6% in the controls) and an increase in the length of incisors in females (14% vs. 2%). There were other seemingly treatment related effects whose biological significance is not well understood such as a decreases in read adhesive substance in females (12% vs. 48% in the controls, and a decrease in skin calluses in males (0% vs. 8% in controls).

At 1300 ppm, there were decreased body weights in both sexes from week 64 to the end of the study in males (↓4-20%), and from week 32 to the end of the study in females (↓3-20%). Absolute organ weights at necropsy were reduced in the males for brains (↓4%), and epididymides (↓55%). Organs with increased statistically significant weight values were the liver (↑22%) and kidneys (↑11%). Absolute organ weights in females which were statistically significantly reduced were the brain (↓6%), spleen (↓73%) and adrenals (↓23%). Gross examination found reduction in testes softening compared to controls (46% vs. 7%), with small increase in the incidence of benign tumor masses in the 1300 ppm group (97% vs. 85% in the controls). Epididymide softening was significantly elevated (97%) when compared to controls (77%). Significant opacity of the eyes was noted at both sexes (78%) and (97%) compared to controls in males and females respectively (9% and 8%). For animals carried to termination at 104 weeks, pituitary masses were significantly reduced (7%) in males at when compared to controls (45%). Female macroscopic examination revealed changes including decreases in eye discharge (2% vs. 24% in the controls), decreased mammary gland hypertrophy (0% vs. 16% in the control), spleen enlargement (0% vs. 13% in the control), and decreased thyroid masses (0% vs. 13% in the controls).

For males at 1300 ppm, mammary gland cysts were reduced (0% vs. 34% in controls), increases were reported in striated muscle atrophy (90% vs. 11% in controls), nasal cavity rhinitis (61% vs. 34% in controls), liver centrilobular hypertrophy (93% vs. 0% in controls), chronic nephropathy of kidneys (73% vs. 25% in controls), atrophy of epididymides (100% vs. 14% in controls), seminal vesicle atrophy (97% vs. 0% in controls), coagulating gland atrophy (97% vs. 0%), prostate atrophy (93% vs. 0% in controls), thyroid increased small-sized follicles (93% vs. 0% in controls), cataract of eye (100% vs. 9% in controls), retinal atrophy (97% vs. 9% in controls) and adrenal hypertrophy of the zona fasciculata/ reticularis cells (2% vs. 0% in controls). Smaller numbers of positive occurrences were exhibited in those found dead and killed in extremis for the following fatty changes in liver, liver cell hypertrophy, increased # small-sized follicles of thyroid, hypertrophy of adrenal zona cells, and cataract of the eye.

Neoplastic lesions were examined microscopically in animals at 104 weeks of treatment and males exhibited an increase in mammary gland fibroadenomas of 5 vs. 1 in controls. Thyroid C-cell adenomas were reduced to 6 vs. 16 in controls and testicular interstitial cell tumors were increased to 41 vs. 33 in controls. When the additional animals were found dead are added, the totals become: thyroid C-cell adenomas 6 **/ 20 when compared to controls. The various individual tumor types (all benign) were not increased in females, but were significantly reduced in incidences: pituitary gland anterior adenoma (4** vs. 20 in controls), mononuclear leukemia (1** vs. 9 in controls and 1** vs.9 in controls) in both the 350 and 1300 ppm groups, and thyroid gland C-cell adenoma (3** vs. 13 in the controls). The biological significance of the decreases in these incidences due to treatment is unclear.

Testicular interstitial (leydig) cell tumors had an increased incidence of 98% and 94% vs. 82% in controls at 350 ppm and 1300 ppm. There was one male at the high dose level that was cannibalized and lost to analysis for this parameter.

At 350 ppm there were numerous effects on clinical signs, body weights, as well as macro- and microscopic changes that were considered treatment-related and adverse. Regarding clinical signs, there was an increased incidence of eye opacity in males (18% vs. 8% in the control). Both males and females lost body weight starting from week 72 to the end of the study (4-7% in males), and from week 32 to the end of the study (3-10% in females). Epididymide weights were reduced (↓35%).

Gross examination found a reduction in testes softening (24%) compared to controls (45%), with a small increase in the incidence of benign tumor masses (100%) compared to controls (86%). Epididymide softening was significantly elevated (97%) when compared to controls (77%). Microscopic evaluations of non-neoplastic lesions in males at 104 weeks of treatment, showed reductions in mammary gland cysts (9.7%) compared to controls (34%). Males also exhibited increased eye opacity (20%) vs. the controls (9%). Based on the statistical evaluation of the male tumor incidences, the conclusion is that the chemical in the diet for 104 weeks induces additional testicular interstitial cell tumors in the treated male rats at 350 ppm of pyrifluquinazon (98% vs. 82% in the controls).

There were no treatment-related effects in the 100 ppm group. Rats at 100 ppm did not differ from controls in regards to body weights or food intake throughout the study.

This study satisfies the guideline for a carcinogenicity study in rats (870.4200). This compound seems to cause an increase in testicular tumor incidence as well as numerous other effects on the male reproductive system, as well as decreases the pituitary mass which is consistent, with the proposed mode of action for an anti-androgenic compound. It is worth noting that even though the compound seems to cause a treatment related increase in leydig cell tumors, these tumors were not considered treatment related by the CARC due to the high background incidence of leydig cell tumors in F344 rats (TXR#0056339).

In females there are was also an increased incidence of hyperplasia in the uterus. The pituitary gland as well as the gonads, are the target organs at the mid-dose. At the high dose, the liver, thyroid, kidney and eyes are also target organs. The biological relevance of some of the effects reported is not clear such as decreased mammary gland cysts in males, as well as decreased eye discharge. Eye discharges are typically seen as some sort of eye injury. It is interesting that the compound slightly decreases female brain weights and also spleen weights in females which is a potential indicator of immunotoxicity. Upon reviewing the raw data, the thymus weight was not measured, so there is no way to correlate this effect with the changes seen in the spleen.

The LOAEL is 350 ppm (equivalent to12.5 / 16.4 mg/kg/day in males/females), based upon decreased body weights in both sexes. In males, there was a decrease in epydidymide weights, increased epididymide softening, decreased testes softening and increased testes mass, increased incidences of testicular atrophy, seminal vesicle atrophy, coagulating gland atrophy, prostate atrophy, and an increased incidence of eye cataracts and interstitial tumors in the testes. In females there was bile duct hyperplasia, decreased zymogen granules and focal acinar cell atrophy in the pancreas, tubular basophilic changes in the kidney, dilation of the uterine horn and retinal atrophy, and spleen hematopoeisis. The NOAEL is 100 ppm (equivalent to 3.53/ 4.51 mg/kg/day in males/females).

The study is considered to be acceptable only as a carcinogenicity study. The additional parameters needed for chronic toxicity testing were carried out in the guideline chronic rat study.

C. STUDY DEFICIENCIES: No deficiencies were noted.